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ABSTRACTS

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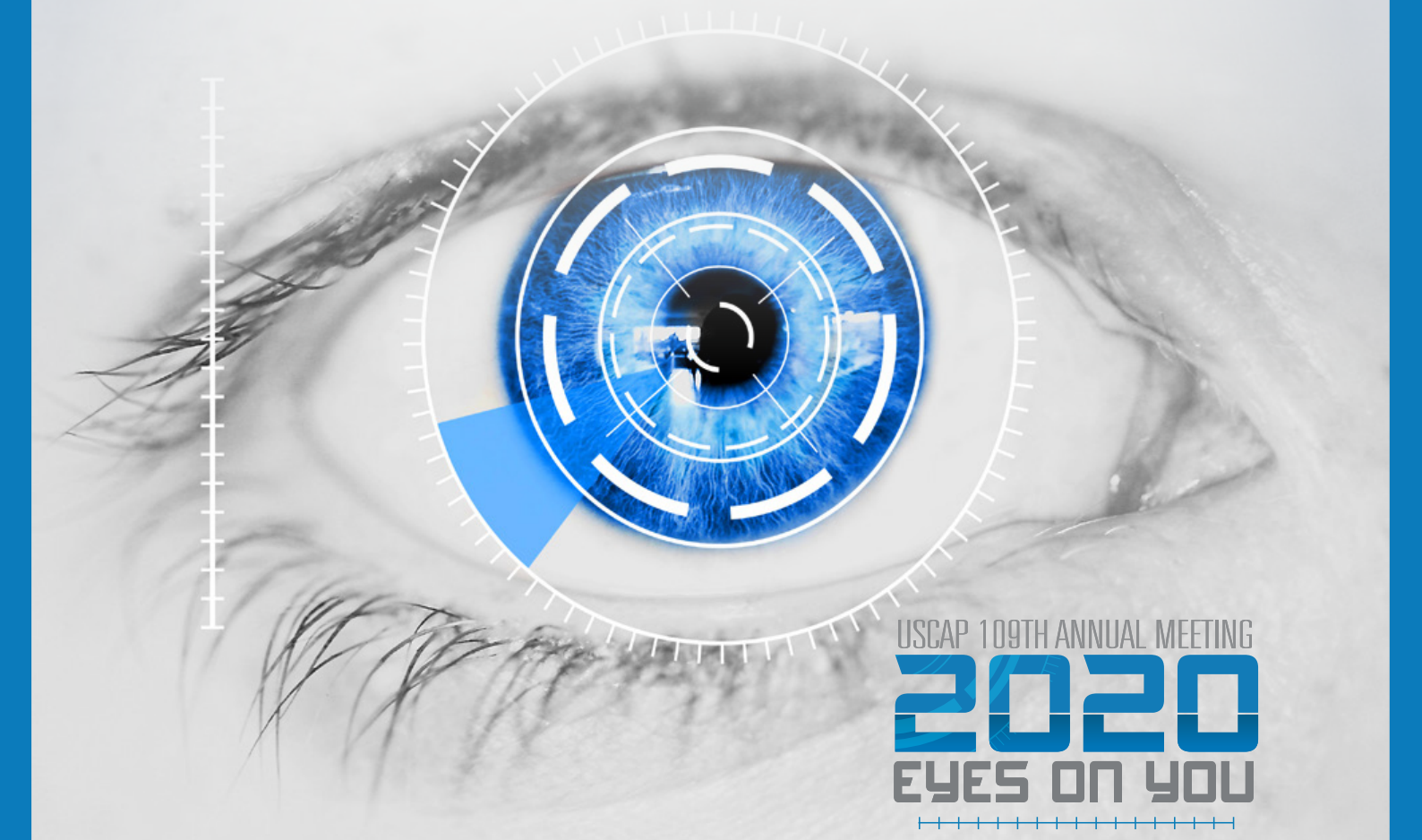

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110 Histopathologic Review, Comorbid Characteristics and Clinical Outcomes of Primary and Secondary Breast Angiosarcoma: A Multi-Institutional Study

Evi Abada[1], Kunil Raval[1], MHD Fayez Daaboul[2], Zhifei Zhang[3], Joseph Trak[1], Saivaishnavi Kamatham[1], Nabil Rahoui[1], Ibrahim Tsolakian[4], Rouba Ali-Fehmi[1], Sudeshna Bandyopadhyay[5]
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Disclosures: Evi Abada: None; Kunil Raval: None; MHD Fayez Daaboul: None; Zhifei Zhang: None; Joseph Trak: None; Saivaishnavi Kamatham: None; Nabil Rahoui: None; Ibrahim Tsolakian: None; Rouba Ali-Fehmi: None; Sudeshna Bandyopadhyay: None

Background: Breast angiosarcoma (BAS) is an aggressive neoplasm with poor prognosis accounting for about 1% of all breast malignancies. It can be classified as either primary (de novo) or secondary BAS (status post radiation). We aimed to study the histopathologic, comorbid characteristics and clinical outcomes of this rare disease.

Design: We retrospectively reviewed databases from two institutions in order to identify patients diagnosed with BAS between 1990 and 2019. Clinical data for these patients was obtained from electronic medical records.

Results: Nineteen patients with BAS were identified from the databases. Of these, 5 (26%) cases were primary BAS and 14 (74%) cases were secondary BAS. The mean age at diagnosis for primary and secondary BAS were 45 (range: 23-71) and 61 (range: 33-84) years respectively. The mean time to secondary BAS development following radiation therapy was 74.6 (range: 24-144) months. The mean follow up period was 23 months (range: 1-108). Disease recurrence was seen in 1 (20%) and 5 (36%) cases of primary and secondary BAS, respectively. 3 (60%) and 2 (14%) patients, with primary and secondary BAS respectively, died of disease. The mean duration from diagnosis to death was 16.8 months (range: 15-29) and 12.5 months (range: 5-20) in primary and secondary BAS, respectively. Patient characteristics including comorbidities and histopathologic descriptors are summarized in Table 1.

Table 1: Patient Characteristics and Prognosis of Primary vs Secondary Breast Angiosarcoma (BAS)

Patient Characteristics	Primary BAS (n=5; 26%)	Secondary BAS (n=14; 74%)
Race		
Caucasian	3 (60%)	8 (57%)
African American	2 (40%)	6 (43%)
Comorbidities		
Overweight/obese	1 (20%)	10 (71%)
Diabetes	1 (20%)	7 (50%)
Hypertension	0	7 (50%)
Smoking	0	4 (29%)
Histopathologic descriptors		
Mean tumor size	8.9 cm (range: 0.5-28)	3.3 cm (range: 0.5-13)
Low grade	2 (40%)	5 (36%)
Intermediate grade	1 (20%)	2 (14%)
High grade	2 (40%)	7 (50%)
Tumor necrosis	2 (40%)	3 (21%)
Mitotic count >10/10HPF	2 (40%)	5 (36%)
Lymphovascular invasion	2 (40%)	3 (21%)
Lymph node metastasis	2 (40%)	1 (7%)

Conclusions: Although no histologic differences were identified between primary and secondary, secondary BAS appears to have a poorer outcome with higher recurrence rates and shorter survival times. Comorbidities such as obesity, diabetes, hypertension and smoking were strikingly more prevalent in patients who developed secondary BAS, which may have prognostic implications.

111 Clinicopathologic Characteristics and Outcome Descriptors of Metaplastic Breast Carcinoma: An Academic Tertiary Institution's Experience

Evi Abada[1], MHD Fayez Daaboul[2], Saivaishnavi Kamatham[1], Joseph Trak[1], Nabil Rahoui[1], Ibrahim Tsolakian[3], Kingsley Ebare[4], Sudeshna Bandyopadhyay[5], Rouba Ali-Fehmi[1]
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Disclosures: Evi Abada: None; MHD Fayez Daaboul: None; Saivaishnavi Kamatham: None; Joseph Trak: None; Nabil Rahoui: None; Ibrahim Tsolakian: None; Kingsley Ebare: None; Sudeshna Bandyopadhyay: None; Rouba Ali-Fehmi: None

Background: Metaplastic breast carcinoma is an aggressive form of breast cancer with poor prognosis, accounting for approximately 1-2% of all breast cancers. We aimed to study the clinicopathologic characteristics and outcomes of this rare disease in our patient population.

Design: This is a retrospective study of patients who were diagnosed with metaplastic breast carcinoma between 2000 and 2019. Clinical data was obtained from electronic medical records. Bayesian Cox Proportional model method was used to determine association between survival and several clinicopathologic variables and hazard ratio (HR) > 1 were considered significant.

Results: Of the 135 patients, 47 (34.8%) were Caucasian, 76 (56.7%) were African-American, and 12 (8.9%) belonged to other ethnicities. Median age at diagnosis and tumor size were 57 years (range: 27-92) and 3 cm (range: 0.5-21.5), respectively. At diagnosis, 100 (74.1%) cases were triple negative tumors and 122 (90.4%) cases were grade III tumors. 73 (54.1%), 37 (27.4%), and 57 (42.2%) cases had non-squamous histology, vascular invasion, and stage II disease, respectively. 21 (15.6%) cases had multi-focal disease. Ductal carcinoma in-situ (DCIS) and fibrocystic breast disease were seen in 56 (41.5%) and 26 (19.3%) cases, respectively. 78 (57.8%) patients were obese (BMI>30), and of those, 53 (68%) were African Americans. During a median follow-up of 24 months (range: 0-221), 107 (79.3%) patients were alive. However, 14 (10.4%) deaths were reported with a median time-to-death of 17.5 months (range: 1-169). Of the 14 patients that died, 13 (93%) were African-American and this was statistically significant with a HR of 11.14 (95% CI:1.13-131.6). Multivariable analysis also showed significant association between tumor size and overall survival (HR: 1.31; 95% CI: 1.17-1.49). Disease recurrence was reported in 22 (16.3%) patients; however, this was not associated with tumor grade or stage at presentation.

Conclusions: Metaplastic breast carcinoma is an aggressive disease and patients with a larger tumor size at presentation have worse overall outcomes. Results from our patient population with metaplastic breast carcinoma suggest that African-American women have worse survival compared to women of other races with similar characteristics. Characteristics such as age, obesity, grade, triple negative status, multifocal disease, presence of vascular invasion, and histologic morphology have no effect on overall survival.

112 9p24.1 Amplification Encoding JAK2, PDL1, and PDL2 in Treatment Naïve Triple Negative Breast Cancer in African American Females

Ali Afsari[1], Lori Koslosky[2], Luisel Ricks-Santi[3], Karen Anderson[4], Michael Barrett[5], Yasmine Kanaan[6], Tammey Naab[7]
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Disclosures: Ali Afsari: None; Karen Anderson: *Primary Investigator*, Co-inventor on patent submission on 9p24.1 detection

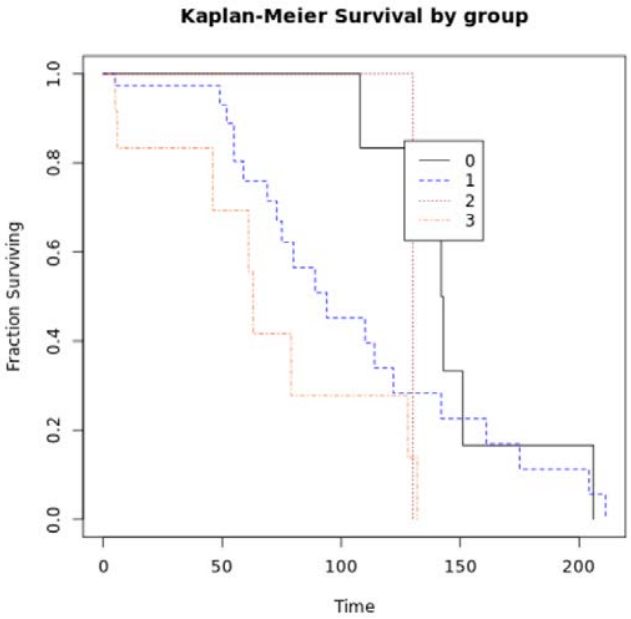
Background: 10-24% of invasive breast cancers (BC) are triple negative (TN), occur with increased frequency in younger African American (AA) women, are clinically aggressive with unfavorable outcome, and have no targeted therapy other than PARP inhibitors. Using array CGH of TNBC, "PDJ" amplicon on chromosome 9p24.1 including PD1-L1, PD-L2 and JAK2 has been shown to be enriched in a subset of TNBC with transcriptional upregulation of JAK2 and PD-L1. The objective was to assess PDJ amplification in breast and lymph node tissue from chemoradiation naïve AA women with TNBC and to correlate with disease-free survival and overall survival.

Design: Tissue microarrays (TMAs) were constructed from FFPE tumor blocks from primary TNBC (87) and axillary lymph nodes (15) in AA women. 2 separate 1 mm cores representing each case was scored. A novel multiplexed fluorescence in situ hybridization (FISH) combined probe (Metasystems) mapping to 9q22 (FITC), 9q34.1 (Texas Red), and a combined JAK2/9p24.1 (PDJ) (TAMRA) was the enumeration probe set used to determine copy number alterations of the PDJ breakpoints. Ratio of PDJ out of 50 cells scored was calculated to determine amplification status. Ratios of PDJ to 9q22 and PDJ to 9q34.1 were calculated to distinguish polyploidy from amplification. Due to significant intratumoral heterogeneity, PDJ signal >4.0, PDJ/9q22>2 and/or PDJ/9q34.1>2 were calculated in the top 10% of cells based on copy number. Survival data and data regarding distant control were gathered. Overall survival and disease-free survival were analyzed using Kaplan-Meier method and compared using log-rank test. Patients were divided into groups according to their amplification status and univariate Cox regression modeling was performed to determine impact of amplification on survival. Statistical analysis was performed using SPSS ver. 21.0 (IBM Inc., Armonk, NY, USA) and statistical significance was achieved with a p-value ≤ 0.05.

Results: 48% (42/87) breast and 80% (12/15) nodes showed PDJ amplification; in 3 of 4 cases with paired breast, only the node showed amplification. 83% (35/42) had PDJ>4. Analysis of 64 cases revealed p=0.055 for overall survival with poor survival linked to an increasing number of amplifications.

This figure measures the number of amplification present. 0=no amplifications 1= 1 amplification 2= 2 amplifications 3= 3 amplifications Chi square= 7.585933

Figure 1 - 112



Conclusions: 9p24.1 (PDJ) amplification encoding JAK2, PDL1 and PDL2 is present in TNBC in AA females (AAF). JAK2 inhibitor may be a consideration for targeted therapy of AAF with TNBC, especially if nodes are positive. Testing on nodes may be useful.

113 Comparison of Biomarkers for Predicting Therapeutic Effects in HER2-Positive Breast Carcinomas

Momoko Akashi[1], Rin Yamaguchi[2], Hironori Kusano[1], Miki Yamaguchi[3], Jun Akiba[4], Tatsuyuki Kakuma[1], Maki Tanaka[5], Yoshito Akagi[6], Hirohisa Yano[1]

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Disclosures: Momoko Akashi: None; Rin Yamaguchi: *Grant or Research Support*, Chugai Pharmaceutical Co.; Hironori Kusano: None; Miki Yamaguchi: None; Jun Akiba: None; Tatsuyuki Kakuma: None; Maki Tanaka: None; Yoshito Akagi: None; Hirohisa Yano: None

Background: The recommended neo-adjuvant chemotherapy (NAC) for HER2-positive breast carcinomas is combined chemotherapy and HER2-targeted therapies. Although the pathologic complete response (pCR) rate is high at 51%, therapeutic responses are not homogenous. For personalized therapy, investigation of new biomarkers is required to improve pCR rates. Estrogen receptor (ER) expression is reportedly correlated with pCR in HER2-positive breast carcinomas, while basal marker expression is related to worse prognosis in ER-negative HER2-positive breast carcinomas after NAC. Previously, we reported that high androgen receptor (AR) expression is useful to predict therapeutic response in HER2-positive breast carcinomas. In this study, we examined the relationship between multiple biomarkers including AR and therapeutic effects in HER2-positive breast carcinomas.

Design: The study cohort comprised patients with HER2-positive invasive breast carcinoma undergoing surgery after NAC (±HER2-targeted therapies) between 2007 and 2017. ER, AR, and basal marker (CK14, CK34βE12, EGFR) expression was assessed by immunostaining of pre-NAC biopsy specimens. ER was considered positive when Allred score ≥2, while the other markers were considered positive when Allred score ≥4. pCR was defined as QpCR (quasi pCR; grade 2b [near pCR] + grade 3 [comprehensive pCR]).

Results: A total of 82 eligible patients was identified. Negative ER, positive AR, and basal marker expression was observed in 37 (45.1%), 43 (52.4%), and 55 (67.1%) patients, respectively. pCR was observed in 40 patients (48.8%) (ER-negative: 22 [59.5%; *P*=0.08]; AR-

positive: 31 [72.1%; *P*<0.001]; basal marker-positive: 31 [56.4%; P=0.05]). In the univariate analyses, negative ER, positive AR, and basal markers were significantly correlated with pCR; only negative ER (*P*=0.03) and positive AR (*P*<0.001) were correlated with pCR in multivariable logistic regression analyses. pCR was observed in 16 (80%) (*P*=0.006) ER-negative AR-positive and 15 (65.2%) (*P*<0.001) ER-positive AR-positive patients. pCR rate was significantly better in AR-positive patients regardless of ER expression.

Conclusions: Multivariate analysis revealed that ER and AR are useful predictors of therapeutic effect compared with other markers, and AR expression was a better significant predictor than ER expression. AR assessment can also determine more responsive ER-positive patients, who have poorer pCR than ER-negative patients with HER2-positive breast carcinomas.

114 Validation of the Revised 8th AJCC Breast Cancer Prognostic Staging System: Analysis of 5,321 Cases from a Single Institution

Rana Aldrees[1], Kui Zhang[2], Gene Siegal[1], Shi Wei[1]
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Disclosures: Rana Aldrees: None; Kui Zhang: None; Gene Siegal: None; Shi Wei: None

Background: The Anatomic Stage Groups (ASG) have been arguably most powerful in predicting breast cancer (BC) outcomes. Recognizing the prognostic influence of histologic grade and ER/PR/HER2 status, the 8th AJCC mandates their incorporation and consolidation into the newly established prognostic stage groups (PSG). The subsequent observations of investigators' incapability to assign up to 13.6% of BCs in the initial staging scheme have resulted in a major revision in the PSG table. The updated staging system provides pathological and clinical prognostic stage tables (PPSG/CPSG), with the former only used for patients having surgical resection as the initial treatment, and the latter for all patients. Given the increasing use of neoadjuvant therapy, PPSG cannot be assigned in a significant proportion of BCs. In this study, we sought to validate the CPSG in a cohort of 5,321 BCs, the largest of its kind from a single center.

Design: All BCs diagnosed at the authors' institution between 1998-2018 were included. Analysis of BC-specific survival (BCSS) was performed using the Kaplan-Meier method and the log-rank test.

Results: Of 8152 invasive BCs diagnosed in the study period, 5,321 had all required elements for CPSG, received standard of care therapy and thus were included in the analyses. The median follow-up was 4.6 years. Compared to ASG, application of CPSG assigned 15% and 25% of cases to higher and lower stage groups, respectively. The grade change was mostly observed in ASG IIA (29%) and IIB (22%). 7.2% of cases changed more than one stage group from ASG. CPSG provided an improved overall discriminating power in predicting BCSS when compared to ASG (X2=2739 vs. 2570, both P<.0001). Pairwise comparisons using the Cox proportional hazard model provided additional evidence of superiority for CPSG. The differences between IA and IB, IB and IIA, IIIA and IIIB as well as IIIB and IIIC were not significant for ASG. In contrast, all categories in CPSG showed a significant difference when compared to their proximate groups, except IB vs. IIA (HR 1.5, P=.06) and IIIA vs. IIIB (HR 1.4, P=.16). Moreover, the hazard ratio for IIIB was greater than that for IIIC in ASG while such scenarios were not seen in CPSG.

Conclusions: The revised 8th AJCC CPSG provided a superior staging scheme for predicting BC prognostic outcomes in all patients receiving standard of care treatment. Further validation using the available data with larger populations and longer follow-up may be needed to refine and improve this table.

115 PDL-1 Testing in Breast Cancer: Should We Be Testing Primary and/or Metastatic Sites?

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Disclosures: Melissa Alexander: None; Yuanxin Liang: None; Nebras Zeizafoun: None; Shabnam Jaffer: None

Background: The Impassion 130 trial established the benefit of adding a checkpoint inhibitor for the first line treatment of metastatic triple negative breast cancer. This led to the FDA approval of atezolizumab and the companion PD-L1 (SP142) Assay, but with no specific indication for testing the primary versus the metastases (met). The goal of our study was to resolve this dilemma.

Design: A total of 33 specimens from 22 patients were identified for which PDL-1 testing was performed based on oncologist request. As per Impassion 130 trial, we used the SP142 Assay with the cutoff of >/= 1% staining for positive immunohistochemical staining.

Results: The 33 specimens were comprised of 17 primary breast carcinomas, 14 mets (liver-5, lymph nodes(LN)-4, lung-3, bone marrow(BM)-1, and brain-1) and 2 chest wall recurrences (CWR). Ten out 14 mets and both CWR were tested synchronously with the older primary mammary carcinoma. Of the 17 primary tumors, 8 were PDL-1 + and 9 were PDL-1 negative. Of the 14 metastases, 4 were PDL-1 + and 10 were PDL-1 negative. When matched with the primary, synchronous findings were found in both the primary and

metastases in 4 cases (3+,1-); the remaining 6 cases were + in the primary and negative in the mets. Both CWR were + but only 1 of the primaries was +.

Conclusions: We found a higher percentage of PDL-1+ in primary tumors (50%) compared to paired mets/CWR. This could be due to greater volume of tissue and immune cells in the primary tumor compared to scantier mets. In some mets sites such as BM and LN, distinguishing the host immune cells from tumor ones was challenging and presented issues with scoring. For these reasons, when evaluating PDL-1 in patients with mets or CWR, an attempt to retrieve the primary block should be done for concurrent PDL-1 evaluation for therapeutic benefit.

116 Prognosis and Categorization of HER2 Fluorescent In-situ Hybridization (FISH) Results in Patients with Invasive Breast Cancer Who Received HER2 Targeted Agents: Analysis of 226 Patients

Mohamed Alhamar[1], Bassam Alkamachi[1], Harshita Mehrotra[1], Jessica Sanchez[1], Daniel Schultz[1], Dhananjay Chitale[2]
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Disclosures: Mohamed Alhamar: None; Bassam Alkamachi: None; Harshita Mehrotra: None; Jessica Sanchez: None; Daniel Schultz: None; Dhananjay Chitale: None

Background: In management of invasive breast cancers (IBC), status of HER2 (ERBB2) gene serves as a strong prognostic predictor & predictive marker of response to HER2 Targeted Agents (HTA). There is significant heterogeneity in response to these agents in HER2-positive cases. Our aim was to determine the distribution of the status of HER2 by FISH in IBC along with its predictive implications.

Design: IBC cases that were tested for HER2FISH & received HTA from 2006-2017 were identified. HER2FISH was interpreted using the ASCO/CAP guidelines at the time of reporting. Cases were grouped as follows: 1) Monosomy (ratio ≥2.0, mean HER2/cell<4.0) 2) Co-Amplified (ratio<2.0, mean HER2/cell ≥6.0) 3) Low amplified (ratio ≥2.0, mean HER2/cell 4.0-5.9) 4) Amplified (ratio ≥2.0-2.99, mean HER2/cell >6) 5) Excessive Amplification (ratio ≥3.0) 6) 2013 Equivocal (ratio<2.0, mean HER2/cell 4.0-5.9) 7) Negative. Outcomes studied were recurrence, metastasis, second breast primary, disease specific survival (DSS) & overall survival (OS).

Results: There were 226 cases, with median age 65 (range 26-98), 58% Caucasians, 24% African Americans, 1.5% others & 16.5% unknown. The median HER2FISH ratio was 2.47 (1.18-21) & HER2 signal/cell 5.71 (2.09-21). Table 1 shows categoric distribution of cases. 60/226-27% patients received neoadjuvant chemotherapy, 139/226-62% hormonal therapy, 187/226-83% adjuvant & 146/226-65% radiotherapy. The median follow up was 207 weeks (4.3-708). Overall, 165/226-73% patients were alive without disease, 17/226-7% alive with disease, 33/226-15% died of IBC & 11/226-5% died due to other causes. 31/226-14% patients developed metastasis, 7/226-3% local recurrence & 4/226-2% second breast primary. The category of HER2FISH status was significantly associated with OS (p<0.05-Figure1), higher HER2 amplification was associated with fewer deaths, possibly reflecting a better response to HTA. Her2FISH status also statistically significantly relates to metastasis (p=0.04-Figure 2) & second primary (p<0.05) but not with recurrence (p=0.09) or DSS (p=0.5) in our cohort.

HER2 FISH category	Cases	Percentage of cases
Monosomy	16/226	7%
Co-amplified	10/226	4.5%
Low amplified	57/226	25%
Amplified	45/226	20%
Excessive amplification	76/226	34%
Equivocal (based on 2013 guidelines)	19/226	8.5%
Negative	3/226	1%

Figure 1 - 116

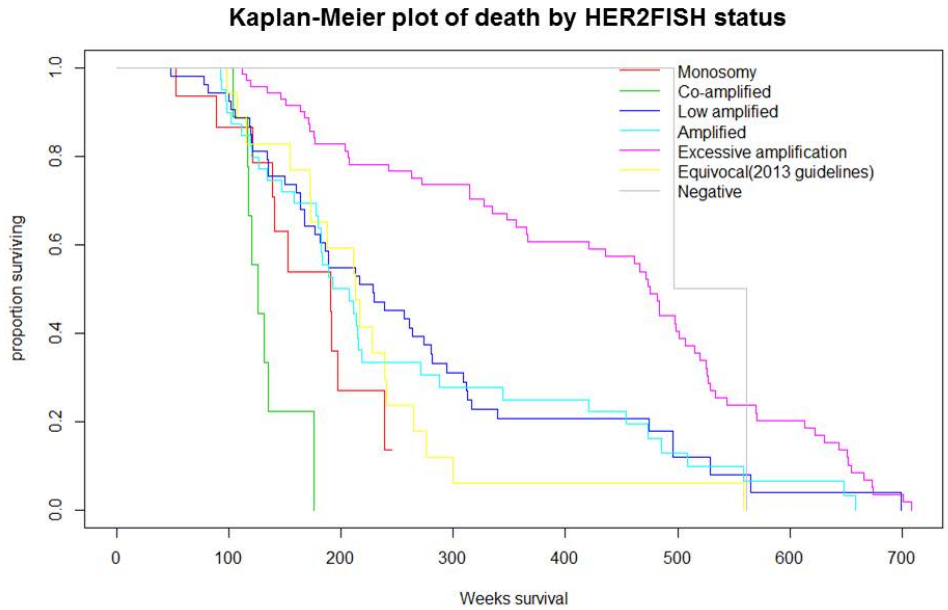
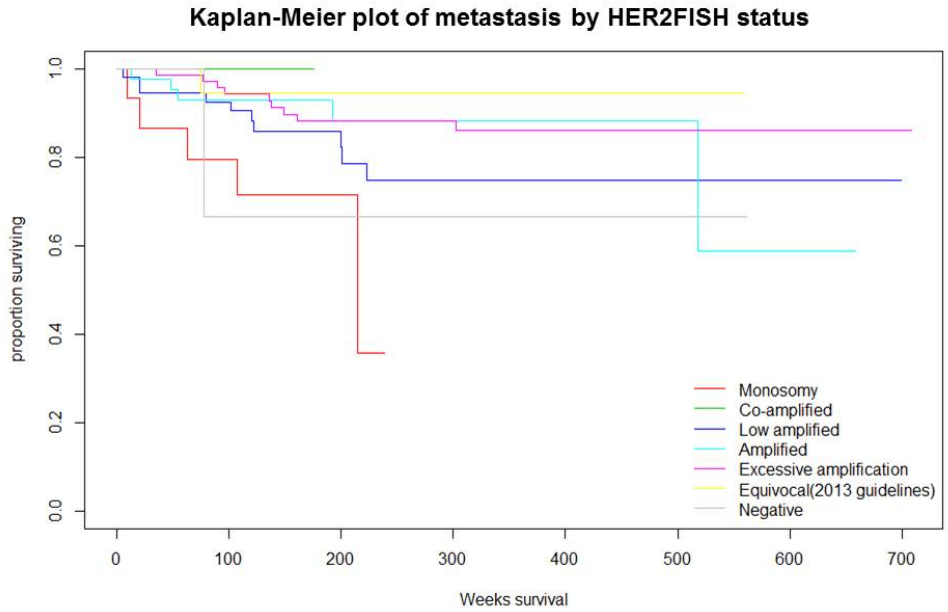


Figure 2 - 116



Conclusions: The current HER2FISH interpretation does not stratify as high or low amplification based on FISH results. In our study, we demonstrate that high HER2 amplification is significantly associated with longer OS; these patients seem to benefit more from HTA. We recommend reporting these categories when assessing HER2FISH in IBC. Larger, prospective longitudinal studies are needed to validate our findings.

117 Evaluation of HER2 Fluorescent In-situ Hybridization (FISH) Status in 274 Patients with Invasive Breast Cancer: Comparison of the Last 3 ASCO / CAP Guidelines for FISH Interpretation and its Effect on HER2 Status Classification

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Disclosures: Bassam Alkamachi: None; Mohamed Alhamar: None; Harshita Mehrotra: None; Jessica Sanchez: None; Daniel Schultz: None; Dhananjay Chitale: None

Background: HER2 gene status as a primary predictor of responsiveness to HER2-targeted therapies in invasive breast carcinomas (IBC), is assessed by in situ hybridization (ISH) for HER2 gene amplification or protein overexpression assessed by immunohistochemistry (IHC). We sought to access the influence of changes in HER2 FISH ASCO /CAP reporting guidelines from 2007, 2013 and 2018 on HER2 status.

Design: This is a retrospective study of patients with IBC, who underwent HER2FISH testing between 2006 and 2017. At our institution, HER2 status is first determined by HER2IHC staining. All the HER2IHC equivocal (2+) cases are reflexed to HER2FISH. HER2FISH status was assessed by using Vysis dual FISH probe (Abbott Molecular, Inc., FDA Approved PathVysion HER-2 DNA Probe Kit). A comparative analysis was made based on 2007, 2013 and 2018 ASCO/CAP guidelines to assess HER2 status reclassification.

Results: Complete data were available on 274 patients with equivocal HER2IHC results, 104 (38%) Caucasians, 52 (19%) African American, 4(1%) other, and 114 (42%) unknown. The results are summarized in tables 1-3 below.

Table 1: HER2 status by ASCO/CAP guidelines

HER2FISH status	**2007**	**2013**	**2018**
Negative	0	11	147
Equivocal	169	123	0
Positive	105	140	127
Total	274	274	274

Table 2: Reclassification of HER2 status from 2007 to 2013 guidelines

2007 vs 2013	**2013 ASCO/CAP guidelines**			
	Negative	Equivocal	Positive	Grand Total
2007 ASCO/CAP guidelines				
Equivocal	11	123	35	169
Positive	-	-	105	105
Grand Total	11	123	140	274

Table 3: Reclassification of HER2 status from 2007 to 2018 guidelines

2007 vs 2018	**2018 ASCO/CAP guidelines**		
	Negative	Positive	Grand Total
2007 ASCO/CAP guidelines			
Equivocal	138	31	169
Positive	9	96	105
Grand Total	147	127	274

Conclusions: We observed 27.2% reclassification rate by using 2013 guidelines compared to 2007, lower threshold for positive (from equivocal, 20.7% of patients re-classified to positive and 6.5% to negative HER2 status). In contrast, the 2018 updates eliminated the FISH equivocal category with concomitant Her2IHC correlation and additional scoring of tumor nuclei. Implementing 2018 CAP guidelines led to 100% reclassification of 2007 equivocal cases into in 81.7% negative cases and 18.3% positive for HER2 status. Prospective data on survival is necessary to evaluate impact of 2018 guidelines on outcomes.

118 Combined Florid Lobular Carcinoma In Situ and Invasive Lobular Carcinoma. A Clinicopathologic Study of 105 Cases

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Disclosures: Isabel Alvarado-Cabrero: None; Rafael Estevez-Castro: None; Raquel Valencia-Cedillo: None

Background: The vast majority of Lobular Carcinoma In Situ (LCIS) cases are incidental findings in excision and biopsies performed for other anomalies, however a small subset of both pleomorphic and classic LCIS may be clinically indentifiable due to their associated calcifications or their presentation as a mass lesion. Classic LCIS has long been considered a risk factor for invasive carcinomas, and treatment often consists of life long follow-up with risk-reduction rather than surgery. Pleomorphic LCIS and florid LCIS are typically considered to be more aggressive than classic LCIS, but management remains controversial.

Design: In this study we describe the clinicopathologic features of 105 cases of florid lobular carcinoma in situ (FLCIS). The archives of the Department of Pathology were searched for all cases of FLCIS diagnosed on core surgical excision/mastectomy between 1997 and 2017. All LCIS lacked complete membranous e-cadherin immunostaining and were further classified as: a) pure FLCIS and b) mixed LCIS/ invasive lobular carcinoma. Clinical data was obtained from the medical records.

Results: Median age at diagnosis was 54 years (range 22-79 years). At diagnosis 36 (35%) had a palpable tumor. The disease was discovered by mammography, with microcalcifications in 45 cases (43%), and opacities on mammography in 24 (22.8%). The lesions were initially identified in an excisional biopsy in 82 cases and in a mastectomy in the remaining 23 patients. In 39 (37.1%) of the cases the LCIS could be described as extensive, in that it was evident in multiple sections involved multiple noncontiguous terminal ductolobular units. An associated invasive lobular carcinoma was present in 69 (65.7%) patients. All invasive cancers lacked lymphovascular invasion. Contralateral LCIS or invasive lobular carcinoma was present in 10 cases (9.5%). All extensive LCIS cases were associated with invasive lobular carcinoma

Conclusions: A substantial proportion of cases of florid LCIS (65.7%), was associated with invasive lobular carcinoma. In this study all cases of extensive LCIS were associated with invasive lobular carcinoma. Florid LCIS demonstrates features of direct precursor lesions warranting surgical excision.

119 How Do Pathologists in Academic Institutions Across the United States and Canada Evaluate Sentinel Lymph Nodes in Breast Cancer? A Practice Survey

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Disclosures: Jaya Asirvatham: None; Julie Jorns: None

Background: Recent clinical trials have changed the management of axillary lymph nodes in breast cancer. There is little data on how this has influenced the way pathologists evaluate sentinel lymph nodes.

Design: A questionnaire was sent to 11 Canadian and 33 US academic breast pathologists. 5 questions related to intraoperative evaluation (IOE) of sentinel lymph nodes (SLN) and 9 to permanent sections.

Results: 5 Canadian and 16 US pathologists, from 21 academic institutions (AI) responded. IOE of SLN is performed only for selected cases (mastectomy, post-neoadjuvant, ACOSOG Z11 non-qualifier) in 9 AI, for almost all cases in 8 AI, and not performed in 4 (3 Canadian). 13 AI use frozen sections (FS) alone, 4 use FS and/or cytologic techniques (2 imprints, 1 smear, 1 either).

During IOC, perinodal fat is completely trimmed in 8, not completely trimmed in 7, and variable in 2 (trimmed tissue submitted for extranodal extension (ENE) evaluation). For FS, in 12 AI the entire node is submitted sectioned at 2mm intervals. Practice is variable in the others. In all AI, non-sentinel lymph nodes are entirely processed at 2mm. Preferred plane of sectioning is parallel to long axis in 8 and perpendicular to long axis in 10, 3 variable).

In 9 AI, only a single H&E slide is obtained from the FFPE block. In 12 AI multi-level evaluation is performed (2 H&E- 7 AI; 3 H&E-4 AI; 4 H&E- 1 AI). In 6 AI, levels are between 1-10 µ deep; in 3 AI, between 10-50 µ deep and 2 AI between 100-200 µ. In 11 AI, cytokeratin immunostain is obtained if necessary on a case by case basis (suspicious cells, lobular variant, post-neoadjuvant). Routine keratin immunostain is obtained in 10 AI (only if lobular- 2 AI; only if frozen negative-2 AI). In 3 AI both AE1/AE3 and CAM 5.2 are obtained.

19 pathologists document extent of ENE (2 qualitative). 11 would consider tumor cells in the pericapsular lymphatic space as lymphovascular invasion (LVI) and 10 would consider it isolated tumor cells (ITC).

Conclusions: There are areas of uniformity and dichotomy in practice. Frozen section is the preferred mode of SNL IOE. In most AI the entire SLN is submitted after sectioning at 2mm and this practice extents to evaluating non-sentinel lymph node for permanent sections; extent of ENE is documented. There is a dichotomy in practice with near equal support for routine vs case-by-case multilevel/immunostain evaluation, perpendicular vs parallel sectioning, complete vs incomplete fat removal and tumor in pericapsular lymphatics as LVI vs ITC.

120 Adenosquamous Proliferations: Clarifying Histologic Features in a Series of 237 Complex Sclerosing Lesions

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Disclosures: Emily Bachert: None; Lames Hamoodi: None; Virgilius Cornea: None; Therese Bocklage: None

Background: Adenosquamous proliferations (ASP) are reported to occur in up to 60% of complex sclerosing lesions (CSLs). The nature of ASPs in CSLs remains unclear including definitional histologic features due in part to evaluation in small series only. ASPs are important, as they are proposed to be the precursor to the rare low-grade, triple-negative adenosquamous carcinoma (LGASC). Moreover, the distinction between a prominent ASP within a CSL and an early LGASC is subjective and has been suggested to be artificial.

Design: Our objective was to determine ASP prevalence and quantitatively analyze ASP histologic features. We identified 306 breast specimens from 2000 to 2019 with a diagnosis of “radial scar” or “complex sclerosing lesion.” Three pathologists evaluated adequate cases for: 1) overall CSL size, 2) central nidus size, 3) presence of lymphocyte clusters, 4) nidus stromal composition, and 5) the presence of squamoid cells. Chronic inflammation was defined as an aggregate of greater than ten lymphocytes or plasma cells located within or immediately adjacent to the nidus. Nidus stroma was classified as: hyalinized/sclerotic, cellular/desmoplastic, or mixed. When applicable, the mixed stroma category was further sub-grouped into hyalinized- and cellular-predominant. Criteria for squamoid features included single cells or small groups with eosinophilic cytoplasm and vesicular nuclei visible at 100x magnification.

Results: From the 306 cases identified, 237 unique complex sclerosing lesions/radial scars met diagnostic criteria (158 cases). Patient age ranged from 20 to 81 years (median: 53). The average size of the overall lesion and the central nidus was 5.5 mm and 2.5 mm, respectively. Of the 237 lesions, 120 were positive for lymphoid aggregates (50.8%), and 87 (36.9%) demonstrated squamoid features (Table 1). Cases with a cellular stroma component of at least 50% were significantly more likely to demonstrate squamoid features and inflammation (74/87 and 74/120, respectively; $p < 0.001$).

Table 1: Features of Complex Sclerosing Lesions by Stromal Category.

Stroma Classification	Cases with Inflammation	Squamoid Features
Hyalinized (n = 90)	31 (34.4%)	7 (7.8%)
Cellular (n = 24)	14 (58.3%)	18 (75.0%)
Mixed (n = 79)	51 (64.6%)	46 (58.2%)
Mixed – cellular predominant (n = 18)	9 (50.0%)	10 (55.6%)
Mixed – hyalinized predominant (n = 26)	15 (57.7%)	6 (23.1%)
Total:	120 cases	87 cases

Conclusions: In our large cohort, inflammation is commonly found in complex sclerosing lesions with squamoid features. Our findings negate the notion that prominent lymphocytes are a diagnostic clue to LGASC on limited biopsy material. Discordant with other series, in our cohort, cases with squamoid features were highly significantly associated with a cellular stroma component, suggesting a possible etiologic relationship between activated stromal cells and preneoplastic epithelial cells.

121 Adenosquamous Proliferations in Complex Sclerosing Lesions (CSLs): Analysis of PIK3CA, SOX4, ER and Luminal and Myoepithelial Marker Expression by Immunohistochemistry

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Disclosures: Emily Bachert: None; Lames Hamoodi: None; Dana Napier: None; Virgilius Cornea: None; Therese Bocklage: None

Background: Amplified SOX4 up-regulates the PI3K/Akt pathway in triple negative carcinomas and regulates epithelial-mesenchymal transition. PI3K/Akt pathway mutations occur in ~60% of CSLs and ~100% of low-grade adenosquamous carcinomas (LGASCs). Shared molecular and histologic features in adenosquamous proliferations (ASPs) and LGASC suggest the two entities are closely related.

Design: Due to morphologic and genetic overlap between CSLs and LGASC, we sought to evaluate CSLs and ASPs by immunohistochemistry (IHC) to evaluate 1) IHC features of ASPs and CSLs, and 2) relationship to LGASC by protein expression. IHC stains were performed for PIK3CA, SOX4, ER, p40, and a breast cocktail (p63 and high-molecular weight keratin (CK) for myoepithelial staining plus CK8/18 and CK7 for luminal staining). The IHC panel was also performed on one LGASC.

Results: Thirty four CSLs underwent IHC staining (n = 24). Fourteen cases (41%) had squamoid cells (single cells or small groups visible at 100x magnification with eosinophilic cytoplasm and round or spindle shape) consistent with ASP (Fig. 1). SOX4 demonstrated wild-type (W-T) expression (no significant difference from normal breast tissue) in all cases. PIK3CA showed W-T expression in the CSLs. Interestingly, even in spindle (presumed nascent) squamoid cells, PIK3CA expression was identified (Fig. 2). IHC for p40 highlighted rare squamoid cells in 9 CSLs. Diminished staining for ER in the CSL nidus was identified in 22 cases (65%). The breast cocktail identified 7 CSLs with rare squamoid cells with myoepithelial-only staining and 8 cases with myoepithelial-only staining in ducts. For comparison, the LGASC demonstrated an identical staining profile as the ASPs.

Figure 1 - 121

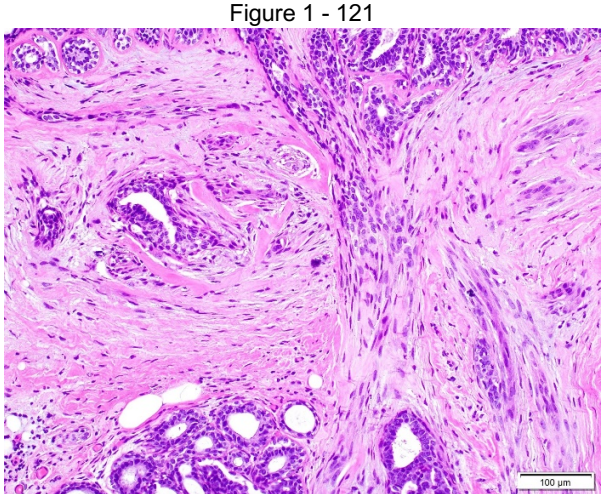
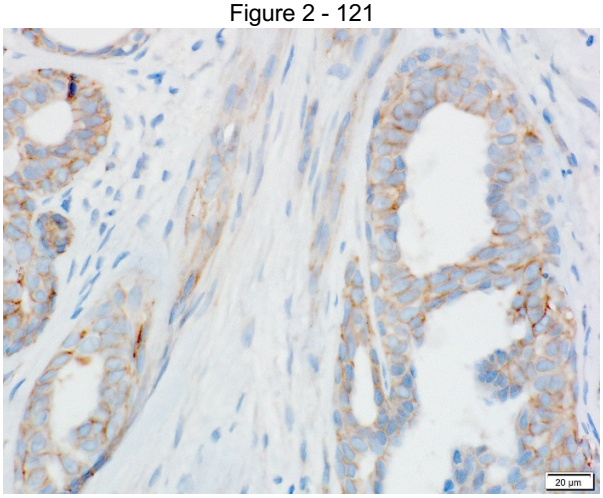


Figure 2 - 121



Conclusions: IHC results parallel morphologic and molecular findings in that ASPs and LGASC stain similarly. PIK3CA generally shows W-T expression. However, PIK3CA expression in squamoid stromal cells distinguishes these cells from surrounding nidus stromal fibrocytes at the IHC as well as histologic level and confirms these cells are distinctly different from surrounding spindle cells. Central epithelial elements lose ER expression further extending the similarity between ASP and LGASC. Absence of ER staining alone should not trigger a diagnosis of LGASC. The breast cocktail IHC reveals a range of reactivity in nidus epithelium in terms of M-E markers (p40, high molecular weight CK and p63). Wild type SOX4 expression in the ASPs and LGASC suggest that it does not play an etiologic role in these lesions.

122 Frequency and Clinicopathologic Features of Hormone Receptor Positive Her2/neu-Amplified Breast Cancer ("Triple Positive Breast Cancers")

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Disclosures: Deyze Badarane: None; Juan Rong: None; Farnaz Hasteh: None; Oluwole Fadare: None; Somaye Zare: None

Background: A subset of breast cancers with overexpression of HER2/neu (HER2) also show immunoreactivity for both the estrogen and progesterone receptors (ER and PR), a group that has been described as “triple positive breast cancers (TPBC)”. Although data is relatively limited on TPBC, emerging data suggests that anti-HER2 treatments are less effective in this group of cancers when compared with their hormone receptor negative (HRN), HER2 amplified counterparts. We aim to characterize the clinical and pathological features associated with TPBC and study their responses to HER2 targeted neoadjuvant therapy.

Design: Clinicopathologic data associated with HER2 positive breast carcinomas from 2014 to 2018 were retrieved from our pathology database. Cases were separated in two groups: TPBC and HRN-HER2-positive. These two groups were compared regarding a variety of clinicopathologic parameters, including patient age, tumor Nottingham grade (1/2+ vs 3+), HER2 IHC status (3+ vs others), HER2 copy number, HER2/CEP17 ratio, and frequency of pathologically-determined complete response to neoadjuvant treatment (PCR).

Results: Of 194 consecutive HER2 amplified breast cancers, 102 (52.5%) cases were TPBC. 93%, 4% and 3% of the TPBC group were invasive ductal, lobular, and mixed carcinomas respectively. TPBC were more often node-positive (56.8% vs 39.1%, p=0.013) and were less often IHC 3+ (57.8% versus 79%, p=0.004), when compared to the HRN-HER2-positive group. In the TPBC group, the median HER2 signals/cell was 7.8 and the median HER2/CEP17 FISH ratio was 3.2. By comparison, for the HRN-HER2-positive group, the median HER2 signals/cell and HER2/CEP17 FISH ratio were 16.6 and 5.8, respectively. Among the patients that underwent neoadjuvant HER2 targeted therapy, PCR was achieved in 12 of 58 (20.6%) TPBC as compared to 29 of 60 (48.3%) HRN-HER2-positive (p=.001). Both groups showed no significant difference in patient age.

Conclusions: In our study cohort, 52.5% of HER2 positive breast carcinomas also expressed hormone receptors. Compared to other HER2 positive subtypes, TPBC are associated with lower mBR grade, higher rate of node-positivity, lower HER2 protein expression, and lower HER2 gene copy number. Notably, these tumors show lower rate of complete pathologic response to HER2 targeted neoadjuvant therapy.

123 Expression of PD-L1 Family Members in Breast Cancer

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Disclosures: Sunil Badve: None; Yesim Gokmen-Polar: None

Background: Expression of the B7 family members such as PD-L1 provide critical co-inhibitory or co-stimulatory signals and have play a major immune modulatory role in cancer. The expression pattern and role(s) of B7 family members, apart from PD-L1, in epithelial neoplasms is not yet fully elucidated. The objectives of this study are to systematically characterize by immunohistochemistry the expression of 5 B7 family members, PD-1, PD-L2, B7.1 (CD80), B7.2 (CD86), and B7-H3 (CD276), in series of breast cancers. Expression of the B7 family m

Design: We analyzed the expression of PD-1, PD-L2, CD80, CD86 and CD276 in a TMA series of 164 breast cancers. Briefly, following antigen retrieval with EDTA, 4um sections were incubated with the PD-1 (1:100, #315M-94; Cell Marque), PD-L2 (1:100, #82723S; Cell Signaling Technology), CD80 (1:200, #AF140), CD86 (1:200, #AF-141-NA) and CD276 (1;200; #AF1027) from R&D systems. Normal pediatric tonsils were used as controls. The staining was analyzed as follows. Negative- less than 1%; positive- greater than 1%. Reverse phase protein analysis (RPPA) data from the breast cancer cohort (n= 741 cases) of the Cancer Genome Atlas (TCGA) was analyzed using the cBioPortal tool.

Results: Expression of CD80, CD86, PD-1 and PD-L2 was essentially restricted to the immune cells of the germinal centers in normal tonsil whereas staining of the crypt epithelium was also observed with CD276. Within the breast, expression of CD86 was cytoplasmic, CD80 was cytoplasmic plus nuclear and CD276 was membranous and peritumoral. The expression pattern was similar in tumors. In cancers, PD-1 was observed only in TILs in 3 cases, PD-L2 membranous expression only was observed in 1 case. In contrast, the expression of CD80, CD86 and CD276 was observed in all cases analyzed, irrespective of the molecular phenotype. None of the cases of breast cancer were positive for PD-1 by RPPA.

Conclusions: PD-1 and PD-L2 expression was rare. RPPA data from the breast cancer cohort in TCGA, which is based on tissue extracts that include stromal and immune cells confirmed the PD-1 expression data. The lack of T-cell co-inhibitory molecules (PD-1 /PD-L2) and the high frequency of expression of co-stimulatory molecules (CD80/CD86/CD276) may explain low response rates in breast cancer for PD-L1 immunotherapies. Alternative approaches are necessary for immune-oncological therapeutic strategies in breast cancer.

124 Androgen Receptor Expression in Breast Cancer: Rate and Patterns of Conversion in Recurrent and Metastatic Disease

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Disclosures: Gabrielle Baker: None

Background: Signaling via the androgen receptor (AR) is known to be involved in mammary gland development; however, its role in breast carcinogenesis and disease progression is incompletely understood. Whereas expression of the estrogen and progesterone receptors as well as overexpression of HER2/neu is routinely evaluated following a diagnosis of invasive mammary carcinoma (IMC), AR status is not typically evaluated outside of the setting of a clinical trial. Data regarding the role of AR expression in IMC is accumulating but only limited data exists regarding the degree to which AR status is conserved with disease progression. As the AR represents a potential therapeutic target, this study seeks to expand knowledge regarding patterns of AR status conversion in the settings of recurrent and metastatic breast cancer.

Design: A cohort of primary IMC with matched, histologically confirmed recurrent and/or metastatic lesions having sufficient archival tissue for further immunohistochemical evaluation was identified. The AR status of all lesions was assessed via a commercially available AR antibody. Data were analyzed as binary results with AR positivity defined as nuclear expression of any intensity in ≥10% of tumor cells.

Results: All patients were female and ranged from 20-82 years of age at initial diagnosis; most had a single recurrent or metastatic lesion available for evaluation (range 1-4; see Table). The majority of primary IMC was AR+ (81%; 38/47) and was of no special type (68%; 32/47). Similarly, the majority of recurrent and metastatic lesions was AR+ (65%; 49/75). A subset of cases demonstrated AR status conversion in the recurrent and/or metastatic setting (18%; 13/74), the majority of these represent conversion from AR+ to AR- (85%; 11/13). Of note, 4/13 cases with AR status conversion represented metastases to the central nervous system (CNS), all of which converted from AR+ primary IMC to AR- metastases.

Site of Recurrent or Metastatic Disease (n)	Sites with AR Status Conversion (n; n from AR+ to AR-)
Axillary lymph node, synchronous (19)	CNS (4; 4)
Bone (10)	Bone (3; 2)
Chest wall (9)	Chest wall (2; 2)
CNS (6)	Liver (1; 1)
Liver (5)	Pleura (1; 0)
Gastrointestinal tract (5)	Soft tissue, non-chest wall (1; 1)
Axillary lymph node, metachronous (4)	Gastrointestinal tract (1; 1)
Lung (4)	
Pleura (4)	
Soft tissue, non-chest wall (4)	
Ipsilateral breast (3)	
Orbit (1)	

Conclusions: In keeping with the existing literature, the majority of primary IMC in this series was AR+. In contrast to several prior reports citing maintenance of AR status in distant disease, in this study a subset of locally recurrent and metastatic disease exhibited AR status conversion with the loss of AR expression most notable in disease metastatic to the CNS. Although the sample size in this study is relatively small, the findings of AR status conversion in metastatic and recurrent IMC is not well documented in the literature and warrants awareness and further investigation.

125 Non-mass Enhancement Lesions of the Breast on Core Needle Biopsy: Outcomes, Frequency of Malignancy and Radiologic-Pathologic Correlation

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Disclosures: Anne Bartels: None; Oluwole Fadare: None; Farnaz Hasteh: None; Somaye Zare: None

Background: Non-mass enhancement (NME) on breast magnetic resonance imaging (MRI) is defined as an area whose internal enhancement characteristics can be distinguished from the normal surrounding breast parenchyma, without an associated mass in the Breast Imaging Reporting and Data System (BI-RADS) lexicon. NME have been associated with a wide variety of benign or malignant lesions. In this study, we evaluated the outcomes of this enigmatic radiological finding at our institution, including the frequency with which malignancy is identified in the associated biopsy.

Design: Our pathology data base was searched for all breast core needle biopsies (CNB) performed for a clinical indication of NME between 1/2010 and 8/2019. Radiologic and pathology findings were reviewed.

Results: A total of 443 cases were identified, comprising 5.5% of all CNBs. The age of patients ranged from 22 to 81 (mean: 51). The pathologic diagnoses were classified as malignant (20.5%), atypical (11.5%), and benign (67.5%). Of the malignant cases, 63 (69.2%) were ductal carcinoma in situ (DCIS) and 28 (30.7%) were invasive carcinomas. Of the invasive cancers, 14 (50%) were invasive ductal carcinoma (IDC), 12 (39.3%) were invasive lobular carcinoma (ILC), and 3 (10.7%) were mixed type carcinomas. 41%, 35% and 24% of the DCIS cases were high, intermediate and low grade respectively. Among the 51 atypical lesions, there were 23 (46%) atypical ductal hyperplasias, 4 (8%) flat epithelial atypias, and 24 (47%) lobular neoplasias (20 lobular carcinoma in situ and 4 atypical lobular hyperplasia). Benign lesions encompassed a spectrum of lesions that included fibrocystic changes, pseudoangiomatous stromal hyperplasia, fibrotic stromal changes, mastitis, and fibroadenomatous alterations.

Conclusions: At our institution, approximately 5.5% of breast core needle biopsies were performed for NME, and up to 32% of these biopsies showed a neoplastic or atypical histologic finding. 20.2% of cases were carcinomas, most of which were in-situ. However, a large proportion of the invasive carcinoma in this setting were ILC (39.3%). The latter is notably disproportionate to the expected frequency of that histotype (approximately 10%) and highlights the need for the pathologist to look for potentially subtle disease in the radiologic setting of NME.

126 Correlation of Mutation Status with Immunohistochemistry in Breast Carcinomas

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Disclosures: Gregory Bean: None; Chieh-Yu Lin: None; Christian Kunder: None; Yunn-Yi Chen: None; Gregor Krings: None

Background: Breast carcinomas (BC) are increasingly analyzed by next-generation sequencing (NGS) for clinical decisions and trial eligibility. The most frequently altered BC genes include *TP53, PIK3CA, PTEN, GATA3, CCND1* and *RB1*. *TP53* is most frequently mutated in triple negative BC and may be prognostic and predictive of therapy response. The p16/ *CDKN2A*-CyclinD1(CycD1)/*CCND1*-Rb axis may also be prognostic and PTEN may be predictive. *GATA3* mutations are often inactivating in luminal BC, which paradoxically have high Gata3 expression. Immunohistochemistry (IHC) for these markers is readily available but correlation with mutation status is unclear. We analyzed IHC and mutation status of commonly mutated genes in a large cohort of BC to determine correlations for clinical utility.

Design: NGS was performed targeting all exons of *TP53* (n=124), *GATA3* (n=130), PTEN (n=103), *CDKN2A* (n=103), *RB1* (n=75) and *CCND1* (n=104). Single nucleotide variants, insertions/deletions, focal amplifications (amp) and deep deletions (DD) were evaluated. Inactivating aberrations (IA) included frameshift, nonsense and splicing mutations and DD. IHC was performed on tissue microarrays of sequenced cases and was evaluable in all. Aberrant staining was defined: p53- negative (neg) or 2-3+ nuclear staining in >90% (DP); Pten, Rb, p16- neg staining; Gata3- neg or cytoplasmic staining; CycD1- DP.

Results: Neg p53 IHC was sensitive (90%) and specific (98%) for TP53 IA, with 18/20 IA associated with neg IHC; 3 IHC neg cases were T*P53* wild-type (PPV 86%, NPV 98%). DP p53 IHC was less sensitive (83%) for *TP53* missense/inframe mutations (MI), with 6 MI cases lacking DP IHC. However, PPV of DP p53 IHC was 100%; no BC without MI *TP53* had DP p53 IHC. Neg Pten IHC was highly sensitive (100%) for *PTEN* IA, but PPV of neg staining was only 44%, with 15 BC without IA being IHC neg. *PTEN* MI (n=2) did not show Pten loss. There was no correlation between *GATA3* IHC and mutation; IA were not associated with protein loss. One *GATA3* splicing mutation showed nuclear and cytoplasmic staining. Neg Rb IHC was not predictive of *RB1* IA (PPV 29%), with 5/7 IHC neg cases lacking IA. DP CycD1 IHC had low sensitivity (62%) for *CCND1* amp and PPV was 47%, with only 8/17 DP cases being amplified. No *CDKN2A* IA were IHC neg (n=4; PPV 0%).

	TP53 (n=124)		GATA3 (n=130)	PTEN (n=103)	RB1 (n=75)	CDKN2A (n=103)	CCND1 (n=104)
Total # mutations	56 (45%)		18 (14%)	14 (14%)	9 (12%)	4 (4%)	13 (13%)
IA	20 (16%)		14 (11%)	12 (12%)	5 (7%)	4 (4%)	0 (0%)
MI	36 (29%)		4 (3%)	2 (2%)	4 (5%)	0 (0%)	1 (1%)
Amp	0 (0%)		0 (0%)	0 (0%)	0 (0%)	0 (0%)	13 (13%)
	Neg IHC/ IA	DP IHC/ MI	Neg IHC/ IA	Neg IHC/ IA	Neg IHC/ IA	Neg IHC/ IA	DP IHC/ amp
Sensitivity of IHC	90%	83%	7%	100%	40%	0%	62%
Specificity of IHC	98%	100%	82%	83%	93%	99%	90%

Positive predictive value (PPV) of IHC	86%	100%	5%	44%	29%	0%	47%
Negative predictive value (NPV) of IHC	98%	94%	88%	100%	96%	96%	94%

Conclusions: p53 IHC is useful to predict mutation status in BC, similar to gynecological cancers. Correlation is less robust between IHC and NGS for *PTEN, CDKN2A, CCND1* and *RB1*. There is no association between *GATA3* IHC and NGS.

127 Genomic Profiling of Primary Angiosarcoma of the Breast

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Background: Angiosarcoma (AS) is a rare mesenchymal neoplasm with aggressive clinical behavior. Primary AS of the breast (PASB) is defined by lack of exposure to radiation therapy for prior cancer. AS from various anatomic sites have been shown to harbor recurrent alterations in *TP53*, MAP kinase pathway genes and genes involved in angiogenic signaling including *KDR* and *PTPRB*. *MYC* amplification is primarily seen in radiation-induced AS. Prior studies largely group AS arising in multiple sites and settings, with heterogeneous results. The pathogenesis of PASB has not been fully characterized. We profiled 10 PASB by capture-based next-generation sequencing (NGS) to determine whether these tumors have characteristic alterations.

Design: Clinicopathologic data was collected for 10 patients, each with no prior history of breast cancer or radiation. DNA was extracted from 9 PASB and matched normal tissue, as well as 1 patient with a known pathogenic *FANCA* germline mutation who presented with bilateral AS 2 years apart. NGS targeted exons of 479 cancer genes, 40 introns and the *TERT* promoter. Single nucleotide variants, insertions/deletions and copy number alterations (CNA) were evaluated.

Results: All patients were female with an average age of 45 years (range 32-64). Tumor size at excision ranged from 2.1 to 13.6 cm (mean 5.2). All patients underwent mastectomy and 6 received adjuvant chemotherapy and radiation. Two patients died of disease. Recurrent genomic alterations were identified in *KDR* (70%), *PIK3CA/PIK3R1* (70%) and *PTPRB* (40%), each at higher frequencies than reported in AS across all sites (21%, p=.004; 21%, p=.004; and 4%, p=.006, respectively). Six cases harbored a *KDR* p.T771R hotspot mutation, and all 7 *KDR*-mutant AS showed evidence of biallelism (4 with loss of heterozygosity, 3 with two aberrations). Three cases were hypermutated (>10 mutations/Mb); both patients who died had hypermutated tumors. Copy number analysis revealed recurrent gains in chromosome 7 (7/10), 8 (5/10) and distal 17q (5/10). The bilateral AS had shared mutations and CNA indicative of contralateral metastasis.
No *MYC*, *TP53*, *RAS* or *FLT4* alterations were detected.

Case #	Laterality	Age	Size (cm)	Rosen Grade	Pathogenic Alterations	Hypermutation Status (# mut/Mb)	Follow-Up (months)
1	R	32	7.9	High	KDR p.T771R, KDR p.Y194delinsCN, PTPRB p.S700*, PTPRB p.S1292I, RASA1 p.S203fs, RASA1 p.R749*	No (2)	NED (7)
2	R	59	2.1	Low	KDR p.T771R, PTPRB p.T1462fs, PIK3CA p.M1004V	No (3)	NED (38)
3	R	31	5.6	Int	KDR p.T771R, KDR p.R725S, PIK3CA p.E545A, GNAQ p.R183Q, PPP2R1A p.R183Q	No (3)	NED (21)
4	R	64	2.8	Low	KDR p.T771R, PIK3CA p.M1043I	No (1)	NED (6)
5	R	49	3	Int	KDR p.T771R	No (3)	NED (9)
6	L	33	7	High	KDR p.T771R, PIK3R1 p.R358P, PIK3R1 c.503-2A>G	Yes (74)	DOD (11)
7	R	35	2.1	Low	KDR p.Q676delinsENQ, KDR p.D731E, PTPRT c.2399+1G>A, PTPRT p.Q1345*, KIT amplification, KDR amplification	Yes (34)	DOD (27)
8	R	49	7.4	Low	PIK3CA p.P104L, BRCA1 p.G1788S	Yes (17)	NED (32)
9	R	54	6	Low	PTPRB p.R566fs, PTPRB p.492_493del, KMT2D p.Q1361fs, PIK3CA p.M1004V	No	NED (36)
9	L	56	2	Low	PTPRB p.R566fs, PTPRB p.492_493del, KMT2D p.Q1361fs	No	NED (9)
10	R	38	13.6	High	PIK3CA p.P539R, ARID1A p.Q562*, KMT2D p.S5404F	No (6)	LFU (9)

mut mutations, Mb megabase, Int intermediate, NED no evidence of disease, DOD died of disease, LFU lost to follow-up

Conclusions: PASB harbor *KDR* alterations more frequently than previously reported and its pathogenesis appears to be distinct from other AS. A subset of tumors is hypermutated, which is possibly associated with worse outcome but may be targetable.

128 Mediator Complex (MED) 7 is Downregulated in High Grade Ductal Carcinoma In Situ (DCIS)

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Background: The Mediator Complex (MED) is a large multi-subunit protein which interacts directly with RNA Polymerase II and transcription factors for general regulation of protein expression. Recently, Mediator Complex 7 (MED7) has been shown to be underexpressed in high grade invasive mammary carcinomas in comparison to lower grade invasive carcinomas. No study has yet investigated the expression of MED7 in ductal carcinoma in situ (DCIS). In our study, we set out to evaluate the biological significance of MED7 in DCIS.

Design: MED7 immunostaining was performed with a rabbit monoclonal antibody (EPR15410, Abcam- Ab187146, Cambridge, UK). The H-score method was utilized for quantifying MED7 nuclear expression in DCIS. This method combines nuclear staining intensity and the percentage of positive cells. Staining intensity (0–3) is multiplied by percentage of positive DCIS cells (0–100) leading to the H-score, with a range of 0–300 for each case. We investigated breast core biopsies with DCIS; 20 were high grade, 14 were intermediate grade, and 15 were low grade. The hormone receptor status of each case was also recorded. GraphPad PRISM 7.0 software was used for statistical analysis.

Results: The mean H-Score for high grade DCIS cases was 146, intermediate grade was 219, and low grade was 228 ($p < 0.0001$, Kruskal-Wallis test). The mean H-score for high grade DCIS cases was significantly lower than that of low and intermediate grade DCIS ($p < 0.0001$ and $p = 0.0004$, respectively, Dunn's test). The MED7 H-Score was significantly lower in ER negative cases compared to that seen in ER positive cases ($p < 0.0001$). Area under the curve (AUC) in the ROC curve for MED7 H-Scores in high grade versus non-high grade DCIS cases was 0.9276 ($p < 0.0001$).

Number of DCIS Cases		
	ER +	ER -
Low Grade DCIS	15	0
Intermediate Grade DCIS	13	1
High Grade DCIS	9	11
Dunn's Test of significant difference, all grades		
	p-Value	Result
Low grade DCIS vs Intermediate grade DCIS	>0.99	*Not significant*
Low grade DCIS vs High grade DCIS	<0.0001	Significant
Intermediate grade DCIS vs High grade DCIS	0.004	Significant
H-Scores of high grade DCIS versus non-high grade DCIS (Mann-Whitney Test)		
	p-Value	Result
High grade DCIS vs Non-high grade DCIS	<0.0001	Significant
H-Scores of ER-positive versus ER-negative DCIS cases (Mann-Whitney test)		
	p-Value	Result
ER+ versus ER-	<0.0001	Significant

Figure 1 - 128

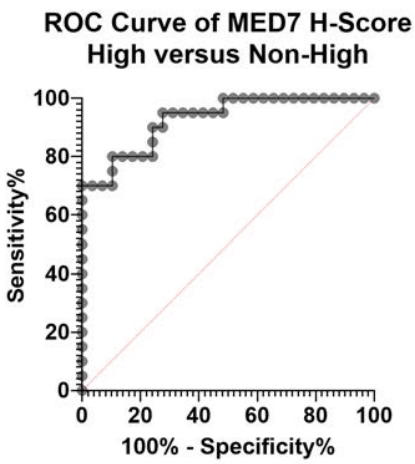
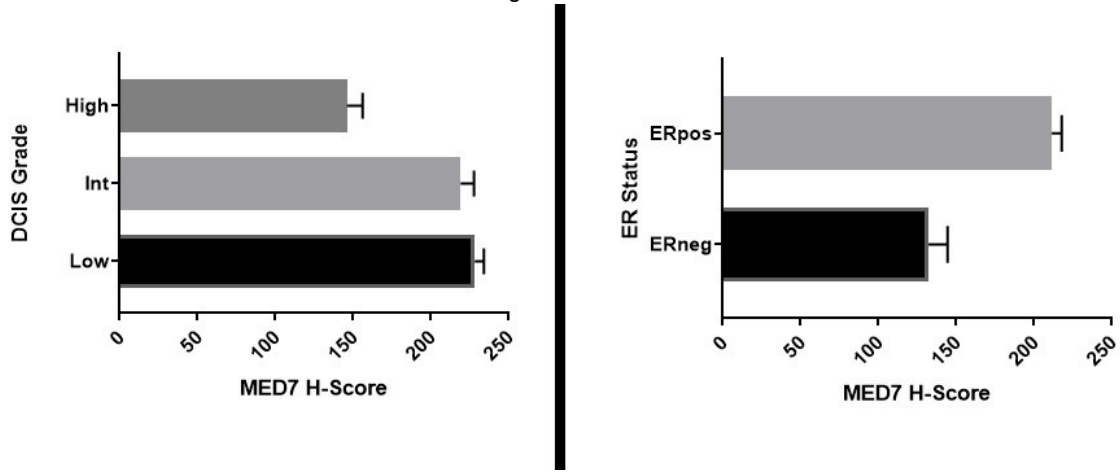


Figure 2 – 128



Conclusions: Our results suggest that MED7 expression is significantly down regulated in high grade and ER-negative DCIS cases, thus implying a significant biological role of MED7 in the progression of DCIS. Taken together, our data establishes MED7 H-score as a tangible tool for evaluating high grade DCIS. Further studies are underway to establish a practical cut-off value for the MED7 H-Score for high grade DCIS. We believe that these important early steps investigating MED7 are crucial in clarifying its important role in the development and progression of breast and other cancers.

129 Triple-Negative Breast Lobular Carcinoma: Morphological, Immunohistochemical, Molecular and Prognostic Characterization - A Comparative Retrospective Study

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Background: Lobular carcinoma is the second most common type of invasive breast cancer. Triple-negative lobular carcinomas (TNLC) which do not express either hormone receptors or HER2, at the time of diagnosis, are extremely rare. Morphological, immunophenotypic and molecular characteristics as well as prognosis of these types of tumors are poorly known. The aim of this work is to better characterize TNLC through comparison with phenotypically close tumors.

Design: We retrieved all TNLCs, at the time of diagnosis, in our databases between 2000 and 2018. Cases were compared to non-lobular triple-negative carcinomas (NLTNC) and to non-triple-negative lobular carcinomas (NTNLC), after being matched according to stage and Elston/Ellis grade. Clinical, morphological and immunohistochemical characteristics, as well as follow-up information's, were collected. A comparative analysis of the different tumors molecular profiles (Exome and RNA sequencing by NGS) was also performed. Analysis of overall (OS) and progression-free survival (PFS) was done according to Kaplan-Meier method.

Results: Thirty-eight cases of TNLC were analyzed. TNLC tumors accounted for 0.1% of invasive breast carcinomas diagnosed. TNLC shared some morphological and immunohistochemical characteristics with both control groups. However, some features were significantly different. Patients with TNLC were significantly older than those with NTNLC ($p = 0.002$) or NLTNC ($p < 0.0001$). In immunohistochemistry, these tumors more frequently expressed SOX10 ($p = 0.0351$) and EGFR ($p = 0.0006$) than NTNLC, but less frequently than NLTNC ($p = 0.0001$). Morphological and immunohistochemical characteristics of the three groups, as well as comparison between groups are summarized in Table 1. Analysis of survival curves did not show a significant difference between the TNLC and the NLTNC. In contrast, TNLC OS ($p = 0.0470$) and PFS ($p = 0.0388$) was significantly worse than NTNLC (Figs 1 and 2).

Table 1. Comparison of clinicopathological and immunohistochemical characteristics of triple-negative lobular carcinomas to non lobular triple-negative carcinomas and non triple-negative lobular carcinomas

Parameters	TNLC	NLTNC	*P*	NTNLC	*P*

	(n=38) (%)	(n=76) (%)		(n=76) (%)	
Age at diagnosis (y) Mean ± SD Median [min-max]	71,7±12,7 74,0 [37-92]	59,2±15,5 57,0 [32-100]	**<0,0001**	63,7±12,6 64,5 [37-91]	**0,0020**
Unifocal tumor	36 (94,7)	69 (90,8)	0,7155	57 (75,0)	**0,0104**
Size (cm) Mean ± SD Median [min-max]	4,1±3,5 2,8 [0,4–15,0]	2,9±1,9 2,5 [0,5–10,0]	0,2906	2,9±2,3 2,0 [0,4-10,0]	0,0953
Tubular differentiation I II III	0 (0,0) 0 (0,0) 38 (100,0)	2 (2,6) 30 (39,5) 44 (57,9)	**<0,0001**	0 (0,0) 1 (1,3) 75 (98,7)	1
Nuclear grade I II III	0 (0,0) 18 (47,4) 20 (52,6)	0 (0,0) 26 (34,2) 50 (65,8)	0,1737	12 (15,8) 47 (61,8) 17 (22,4)	**0,0009**
Mitosis score I II III	29 (76,3) 5 (13,2) 4 (10,5)	24 (31,6) 22 (28,9) 30 (39,5)	**<0,0001**	51 (67,1) 11 (14,5) 14 (18,4)	0,5123
Histologic subtypes Classic Pleomorphic	19 (50) 19 (50)	- -	-	59 (77,6) 17 (22,4)	**0,0028**
Vascular emboli	15 (39,5)	24 (31,6)	0,4023	20 (26,3)	0,1511
Necrosis	6 (15,8)	22 (28,9)	0,1239	3 (3,9)	0,0581
TILs (%) Mean ± SD Median [min-max]	9,1±14,7 5,0 [1-80]	20,7±20,5 15,0 [1-90]	**0,0005**	8,0±11,8 4,5 [1-60]	0,3130
AR (Quick-score) Mean ± SD Median [min-max]	232,9±90,5 270 [0–300]	81,8±109,9 10 [0–300]	**<0,0001**	255,0±65,1 300 [0–300]	0,2096
CK5/6	3 (7,9)	49 (64,5)	**<0,0001**	3 (3,9)	0,3986
EGFR	8 (21,1)	52 (68,4)	**<0,0001**	1 (1,3)	**0,0006**
SOX10	3 (7,9)	36 (47,4)	**<0,0001**	0 (0,0)	**0,0351**
Ki67 (%) Mean ± SD Median [min-max]	16,6±19,6 10,0 [1-90]	38,9±24,9 32,5 [2-90]	**<0,0001**	13,7±10,7 10,0 [1-50]	0,9182
Abbreviations: TNLC: triple-negative invasive lobular carcinoma, NLTNC: non lobular triple-negative carcinoma, NTNLC: non triple-negative lobular carcinoma, AR: Androgen receptor, CK5/6: cytokeratin 5/6, EGFR: epidermal growth factor receptor.					

Figure 1 - 129

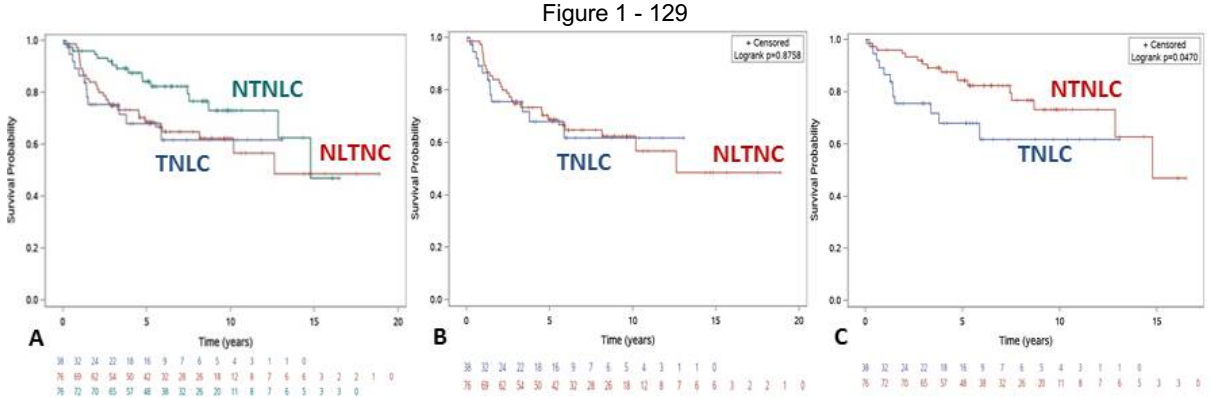

Fig. 1. Comparison of overall survival between triple-negative lobular carcinoma (TNLC), non lobular triple-negative carcinoma (NLTNC) and non triple-negative lobular carcinoma (NTNLC) (**A**), TNLC versus NLTNC (**B**) and TNLC versus NTNLC (**C**).

Figure 2 - 129

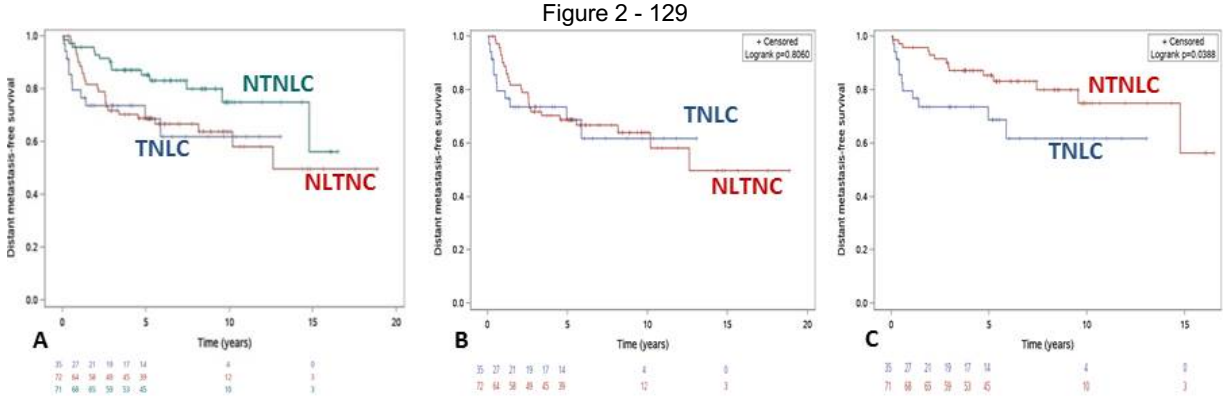

Fig. 2. Comparison of distant metastasis-free survival between triple-negative lobular carcinoma (TNLC), non lobular triple-negative carcinoma (NLTNC) and non triple-negative lobular carcinoma (NTNLC) (**A**), TNLC versus NLTNC (**B**) and TNLC versus NTNLC (**C**).

Conclusions: Results show that TNLC have some distinct clinical, morphological and immunophenotypic characteristics but also share features with NTLNLC and others with NLTNC. At equal stage and grade, TNLC survival is comparable to NLTNC but much worse than NTNLC, despite an identical proliferation index (mitosis, Ki67). Molecular analysis, currently underway and presented during the USCAP 2020 meeting, will attempt to find molecular explanations (mutations, activation of signaling pathway) to explain this prognosis, apart from the absence of hormone receptors expression.

130 Magee Decision Algorithm: An Algorithmic Approach Using Magee Equations and Mitosis Score to Safely Forgo Molecular Testing in Breast Cancer

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Background: Magee Equations™ (MEs) are multi-variable models that can estimate oncotype DX® (ODX) recurrence score and ME3 has been shown to have chemopredictive value in the neoadjuvant setting as a standalone test (PMID: 23503643, 28548119). We recently described a decision algorithm using Magee Equations and tumor mitotic activity score to safely forgo ODX testing (PMID: 30395177).

Design: The current study tests the accuracy of Magee Decision Algorithm (MDA) using a large in-house database. According to the algorithm, if all ME scores are <18, or 18-25 with mitosis score of 1, then ODX testing is not required as the actual ODX recurrence score (RS) is expected to be ≤25 (labeled “do not send”). If all ME scores are 31 or higher, then also ODX testing is not required as the actual score is expected to be >25 (also “do not send”). All other cases could be considered for testing (labeled “send”). We analyzed all ER+, HER2-negative cases sent for ODX testing with available pathology parameters for calculation of all MEs. Two different datasets were utilized-a retrospective database of 1824 cases (years 2007-2015) and 372 prospectively collected cases in the last 3 years sent for clinical ODX testing with a total of 2196 cases.

Results: Of the 2196 cases, 1538 (70%) were classified as “do not send” and 658 (30%) as “send”. The classification accuracy in the “do not send” group was 95.1% (see Table 1). With respect to the “75 discordant cases”, 67 patients were from the retrospective database on which long term follow up was available. Of the 67 cases, 36 received chemo-endocrine therapy, 2 received chemotherapy only, 24 had endocrine therapy alone (mostly an aromatase inhibitor), and 5 did not receive any systemic therapy. The average follow up was 78 months. There were 3 distant recurrences, 2 in patients that received chemo-endocrine therapy and one in a patient who did not receive any systemic therapy. No distant recurrences were recorded in the group that received hormonal therapy alone.

Table 1:

	Do not send- expect high risk	Do not send- expect low risk	Total
Actual ODX RS >25	19	75	94
Actual ODX RS ≤25	1	1443	1444
Total	**20**	**1518**	**1538**

Accuracy of "do not send": 19+1443/1538 = 95.1%

Ability to predict low-risk (≤25): 1443/1518= 95.1%

Ability to predict high-risk (>25): 19/20= 95%

Conclusions: The Magee Decision Algorithm using Magee Equations and mitosis score accurately identifies cases that will not benefit from ODX (or other similar) testing. Such cases constitute ~70% of the routine clinical ODX requests. If Magee algorithm is regularly used in routine practice, then it will save at least $300,000 per 100 test requests. The occasional discordant cases (expected ≤25, but actual ODX RS >25) appears to have an excellent outcome on endocrine therapy alone.

131 Magee Equations™ and Response to Neoadjuvant Chemotherapy in ER+/HER2-Negative Breast Cancer: A Multi-Institutional Validation Study

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Disclosures: Rohit Bhargava: None; Nicole Esposito: None; Siobhan O'Connor: None; Zaibo Li: None; Bradley Turner: None; Ioana Moisini: None; Aditi Ranade: None; Dylan Miller: None; Xiaoxian Li: None; Harrison Moosavi: None; Beth Clark: None; David Dabbs: None

Background: Magee Equations™ (ME) are multi-variable models that can estimate onco*type* DX® recurrence score. Magee Equation 3 (ME3) which utilizes only estrogen receptor (ER), progesterone receptor (PR), HER2 and Ki-67 semi-quantitative results is chemo-predictive in the neoadjuvant setting (PMID: 28548119).

Design: A multi-institutional study involving 8 institutions was undertaken to examine the validity of ME3 in predicting response to neoadjuvant chemotherapy in ER+/HER2-negative breast cancers. The study was limited to stage I-III cases diagnosed in the year 2010 to 2014 in order to facilitate comparison to the original study from the same time-period (PMID: 28548119).

Results: A total of 166 cases met the inclusion criteria. The patient age ranged from 24 to 83 years (median 53 years) with 75 patients (45%) age 50 years or younger. The average pre-therapy tumor size was 3.9 cm, and axillary lymph nodes were confirmed positive by pre-therapy core biopsy in 85 of 166 cases (51%). Pathologic complete response (pCR) was seen only in tumors with ME3 scores >25, with the highest pCR rate in tumors with ME3 score 31 or higher (see table). The pCR rate was not different in patients ≤50 years (n=75) and was again determined by ME3 scores (0% pCR in scores <18, 0% in 18-25, 11% in >25 to <31, and 27% in 31 or higher; p-value: 0.0092). Other Magee Equations (ME1, ME2, and average score) were also evaluated in 159 of 166 cases which showed similar results as ME3 (data not shown). Pre-therapy tumor Nottingham grade also predicted for chemotherapy response (pCR in grade I: 1/20 [5%]; grade II: 3/89 [3%]; grade III: 13/57 [23%]; p-value I & II versus III: 0.0002); however, stratification using ME3 showed the best results. There were no distant recurrences and no deaths in 17 patients with pCR. In the remaining 149 cases with residual disease, ME3 score of >25 was associated with significantly shorter distant recurrence-free survival and showed a trend for shorter breast cancer-specific survival (see figures).

Table 1: Pathologic complete response rate based on Magee equation 3 scores		
Magee Equation 3 scores	Pathologic Complete Response	P-value
< 18 (n=64)	0/64 (0%)	Reference
18 to 25 (n=45)	0/45 (0%)	1.000
>25 to <31 (n = 21)	3/21 (14%)	0.0135
31 or higher (n = 36)	14/36 (39%)	<0.0001

Figure 1 - 131

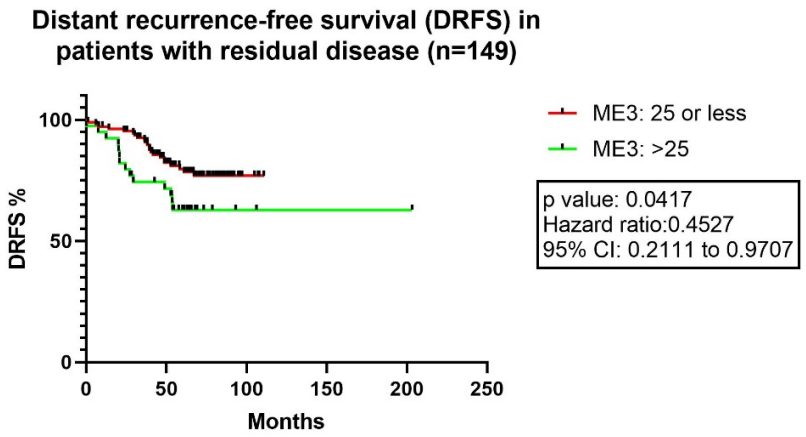
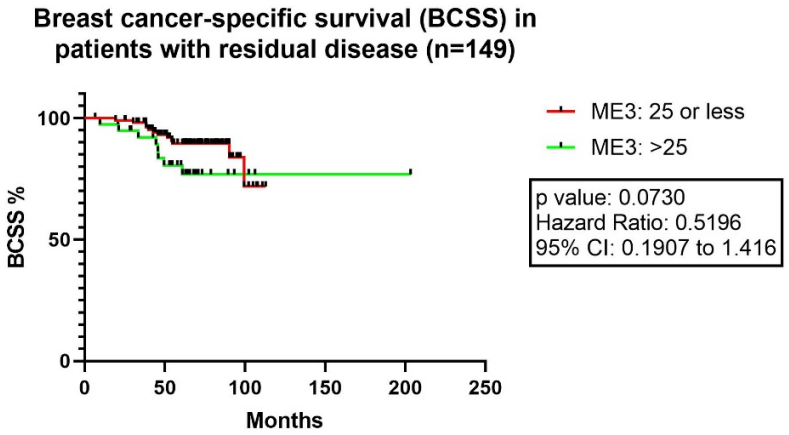


Figure 2 - 131



Conclusions: The results of this multi-institutional study are similar to previously published data from a single institution (PMID: 28548119) and confirms the chemo-predictive value of ME3 in the neoadjuvant setting. Additionally, ME3 may provide prognostic information in patients with residual disease and this should be further evaluated.

132 Breast Cancer in Lynch Syndrome: Clinical and Histopathologic Findings from a Single Institution

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Disclosures: Alexander Blank: None; Holly Pederson: *Consultant*, Myriad Genetics; Erinn Downs-Kelly: None

Background: Lynch syndrome (LS) is an inherited cancer-susceptibility disorder due to pathogenic germline mutations in DNA mismatch repair (MMR) genes, including *MLH1, MSH2, MSH6,* and *PMS2*. Syndromic patients (pts) are known to have increased risk for colorectal, endometrial, ovarian, small bowel, urothelium, biliary tract, and gastric carcinoma (CA). Controversy exists if breast carcinoma (Br CA) should be considered a LS related neoplasm. This study assesses a cohort of known LS pts with Br CA.

Design: Br CA pts with known LS were identified from an institutional registry. Data collected included age at Br CA diagnosis, prior relevant tumor diagnoses, histopathologic features including tumor type and grade, ER, PgR and HER2 status, stage, tumor-infiltrating lymphocytes (TILs). Mismatch repair (MMR) immunohistochemistry (IHC) for MLH1, MSH2, MSH6 and PMS2 was performed and scored blinded to the germline results.

Results: Table 1 collates the data collected. Overall, 50% of Br CA pts were found to have loss of MMR proteins that corresponded to their known mutations. The average age for pts with MMR proficient (MMR-p) tumors was 49.4 (range 42-65) while the average pt age for MMR deficient (MMR-d) tumors was 62. Prior CA diagnoses (colon CA, n= 2 identified 9 and 10 years prior and urothelial CA, n=1 identified 2

years prior) were seen in 60% of the MMR-d group and in 20% of the MMR-p group (endometrial CA, n=1 identified 9 years prior). Tumor morphology was variable in both groups while MMR-d and MMR-p tumors had similar ER, PgR and HER2 status. Stromal TILs in the MMR-d group averaged 45% (range 10-85%) while MMR-p cases averaged 12.5% (range 10-15%). MMR-d tumors were seen most commonly in pts with *MLH1* mutations (3/5), while none of the MMR-p tumors were germline *MLH1* mutated.

Case	Age at Br CA diagnosis	Prior Cancer History	Tumor Morphology, Stage, Grade and Biomarker Status	Stromal TILs	MSH2	MSH6	MLH1	PMS2	MMR Status	Germline Data
1	75	Colon Ca dx 10 yrs prior to breast CA	ILC, NG 2, pT1c pN0 ER+, PgR+, HER2 -	85%	Intact	Intact	Loss	Loss	Deficient	*MLH1*
2	63	Colon Ca dx 9 yrs prior to breast CA	IMC with mixed features, NG 2, pT1c pN0, ER+, PgR+ and HER2-	10%	Intact	Intact	Loss	Loss	Deficient	*MLH1*
3	64	LCIS and ADH dx 12 years prior to breast CA in ipsilateral breast	IMC with mixed features, NG 2, pT1c pN0, ER+, PgR- and HER2-	60%	Intact	Intact	Loss	Loss	Deficient	*MLH1 (*VUS *in MSH6)*
4	63	Urothelial and colon CA dx 2 years prior to breas CA	ILC, pleomorphic and solid type, NG 3, pT1c pN0	20%	Loss	Loss	Intact	Intact	Deficient	*MSH2*
5	45	No prior CA Hx	ILC with treatment effect, NG2, ypT1a ypN2, ER+, PgR+, HER2-	50%	Intact	Intact	Intact	Loss	Deficient	*PMS2*
6	65	Endometrial CA 9 years prior to breast and colon CA diagnosed within same year	DCIS, nuclear grade 3, solid type, pTis (DCIS) pN0, ER+	NA	Intact	Intact	Intact	Intact	Proficient	*MSH2*
7	42	No prior CA Hx	ILC, NG 2, mpT3 pN0 (i+), ER+, PgR+, HER2 -	15%	Intact	Intact	Intact	Intact	Proficient	*PMS2*
8	56	No prior CA Hx	IDC, NOS, NG3, pT2 pN0, ER-, PgR- and HER2-	10%	Intact	Intact	Intact	Intact	Proficient	*MSH6*
9	42	No prior CA Hx	ILC, NG 2, pT1c pN0 ER+, PgR+, HER2 -	10%	Intact	Intact	Intact	Intact	Proficient	*PMS2*
10	42	No prior CA Hx	IDC with mucinous features, NG3, pT2 pN0, ER+, PgR+ and HER2-	15%	Intact	Intact	Intact	Intact	Proficient	*MSH6*

Conclusions: We found that half of Br CAs seen in LS were MMR-d and correlated with known mutations. Higher numbers of TILs were noted in this group and given that PD1/PDL1 inhibitors work in MMR-d tumors, prominent TILs might serve as a potential biomarker of response. The MMR-p group average age of Br CA diagnosis was a full decade younger than the MMR-d group (49.4 vs 62) and although these cancers were not Lynch related, it brings up the importance of vigilant screening in this population. Given that the majority of pts in the MMR-p group had no prior CA history, their germline mutations were identified on multi-panel gene testing owing to the Br CA diagnosis, supporting the broadening use of such assays.

133 Quantitative Digital Immune Profiling Reveals Spatially-Agnostic and Spatially-Constrained Prognosticators in Triple-Negative Breast Cancer

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Disclosures: Jodi Carter: None; Saranya Chumsri: None; Yaohua Ma: None; Jennifer Kachergus: None; Susanna Lauttia: None; Sarah Warren: *Employee*, NanoString Technologies; *Stock Ownership*, NanoString Technologies; Heather Ann Brauer: *Employee*, NanoString; Douglas Hinerfeld: *Employee*, Nanostring; Heikki Joensuu: *Employee*, Orion Pharma; *Consultant*, Neutron Therapeutics; *Stock Ownership*, Sartar Therapeutics; Aubrey Thompson: None

Background: Immune-related biomarkers have emerged as actionable targets in triple-negative breast cancer (TNBC). We used quantitative digital spatial profiling to 1) characterize the immune architecture of distinct tumoral and stromal regions in TNBC and 2) identify spatially-defined prognostic immune biomarkers.

Design: From the FinXX trial of adjuvant fluoropyrimidine therapy, FFPE whole tumor sections from a patient subset with TNBC, matched for treatment arm and outcome (N=44) were assessed with the Nanostring GeoMx™ DSP platform. Using 3-plex immunofluorescence (pan-cytokeratin, CD45, CD68 with SYTO-13 dye), tumor and stroma segments (N=950) were classified as 1) Peripheral Peritumoral stroma (PTS) or 2)Adjacent tumor (PTTu), 3)Intratumoral stroma (ITS) or 4) Adjacent tumor (ITTu) and 5)central tumor with no stroma (CTu). Per segment, digital quantitation (NanoString nCounter®) of 42 immune biomarkers, including immune cell profiling proteins (e.g. CD3, CD4, CD8, CD11c, CD20, CD56, CD68, PD-1; PD-L1), immune drug targets (e.g. VISTA, STING, IDO1), and activation status-related proteins (e.g. ICOS, PD-L2, CD40, CD40L, CD44) was performed. Normalized, differentially-expressed (DE) proteins were evaluated for associations with durable recurrence free survival (RFS)(> 1.5 fold change (FC); p< 0.05).

Results: 20 DE immune proteins were significantly associated with RFS, including 5 “spatially-agnostic” biomarkers (PD-L1, PD-L2, CD20, IDO1, and fibronectin), enriched in ≥ 4 (of 5) tumor and stroma segments, and a “spatially-constrained” subset, restricted to ≤ 3 segment classes (N=15). Among the latter, CD4, CD8, CD11c, CD45, TGFB1 and VISTA were prognostic only in PTTu segments (FC 1.5-1.9); CD44 was associated with RFS only in ITTu. Overall, top up-regulated proteins associated with RFS were PD-L2, CD56 and HLA-DR (FC> 3 in ≥1 segment). Tumor segments had the most prognostic DE immune proteins: PTTu (N=18), ITTu (N=12) and CTu (N=11), with 9 shared proteins (CD20, CD40, CD56, HLA-DR, IDO1, ICOS, beta 2 microglobulin, PD-L1 and PD-L2). Stromal segments had 3 shared RFS-associated DE immune proteins (PD-L2, IDO1 and fibronectin, FC: 1.6-4.0).

Conclusions: With DSP-based quantitative mapping, we identified novel groups of spatially-agnostic and spatially-constrained prognostic immune proteins in TNBC. PD-L2, CD56 and HLA-DR were the top DE proteins and the peripheral tumor had the largest unique protein set. These data add a new dimension to our understanding of the immunobiology of TNBC.

134 Immune Parameters Associated with Survival in Metaplastic Breast Carcinoma

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Disclosures: Xue Chao: None; Peng Sun: None; Lili Liu: None; Jiehua He: None

Background: Metaplastic breast carcinoma (MBC) is a rare histological type of triple negative breast cancer (TNBC), which is not sensitive to adjuvant therapy and have poorer prognosis. MBC is a group of neoplasm exhibiting metaplastic change to squamous or mesenchymal elements. The prognosis predictive significance of tumor infiltration lymphocytes(TILs) has been well demonstrated in breast cancer especially in TNBC. Additionally, the expression of PD-1 and PD-L1 has heterogeneous expression type in diverse tumors. This study aimed to investigate the diversity of immune parameters in different components of metaplastic breast carcinoma.

Design: All the patients diagnosed with MBC from January 2006 to December 2017 were included in our study. Patients with recurrences at diagnosis, previous malignances, immune deficiencies were excluded. The medical records and tumor slides were reviewed. The percentage of stromal TILs, intratumoral TILs, TILs at the invasive margin were evaluated. The stromal TILs were also quantificated. Besides, the quantification of CD4+ and CD8+ TILs using immunohistochemistry were evaluated by digital image analysis (Halo imaging analysis software; Indica Labs, Corrales, NM). The immunohistochemical expression percentage of PD-1 and PD-L1 in tumors and stroma were also analyzed by digital software above. All these variables were re-evaluated separately by two pathologists blind to clinical data. Statistical analysis was performed with t test, chi-square test, univariate and multivariate cox regression.

Results: 61 patients were included in our study. The median age at diagnosis was 50 years (range: 25-81years). The clinicopathological characteristics were listed in Table 1. There is a median follow-up of 48 months (range: 22-163 months). More stromal TILS (>30/0.1mm^2)

in MBC were proved to be associated with longer disease-free survival (DFS) (HR, 0.26; 95%CI, 0.07–0.97), and this trends become stronger in MBC with squamous cell cancer (HR, 0.07; 95%CI, 0.008–0.64). Also, more CD4+ (>25/0.1mm^2) TILs and CD8+ (>7/0.1mm^2) TILs were also associated with longer DFS (CD4: HR, 0.12; 95%CI, 0.02–0.93; CD8: HR, 0.21; 95%CI, 0.05–0.95), . The >1% expression of PDL1 in tumor (HR, 0.19; 95%CI, 0.04–0.85) and >1% expression of PD1(HR, 0.20; 95%CI, 0.05–0.91) in stroma were both associated with longer DFS.

Table 1 Clinicalpathological characteristics

Patients characteristics	**n**	**%**
Histological subtype		
squamous cell carcinoma	**37**	60.66
spindle cell carcinoma	**4**	6.56
chondriod differentiation	**4**	6.56
osseous differentiation	**4**	6.56
fibromatosis-like	**2**	3.28
mixed type	**9**	14.75
With invasive carcinoma with no special type	**32**	52.46
Tumor size(mm)		
0-20	**3**	4.92
21-50	**39**	63.93
>50	**12**	19.67
Lymph node metastasis		
Negative	**47**	77.04
1-3	**12**	19.67
4-10	**2**	3.28
>10	**0**	-
Clinical outcome		
Local recurrences	**5**	8.02
Distant metastasis	**7**	11.48
Total Death	**6**	9.81
Cancer-specific death	**3**	4.92

Figure 1 - 134

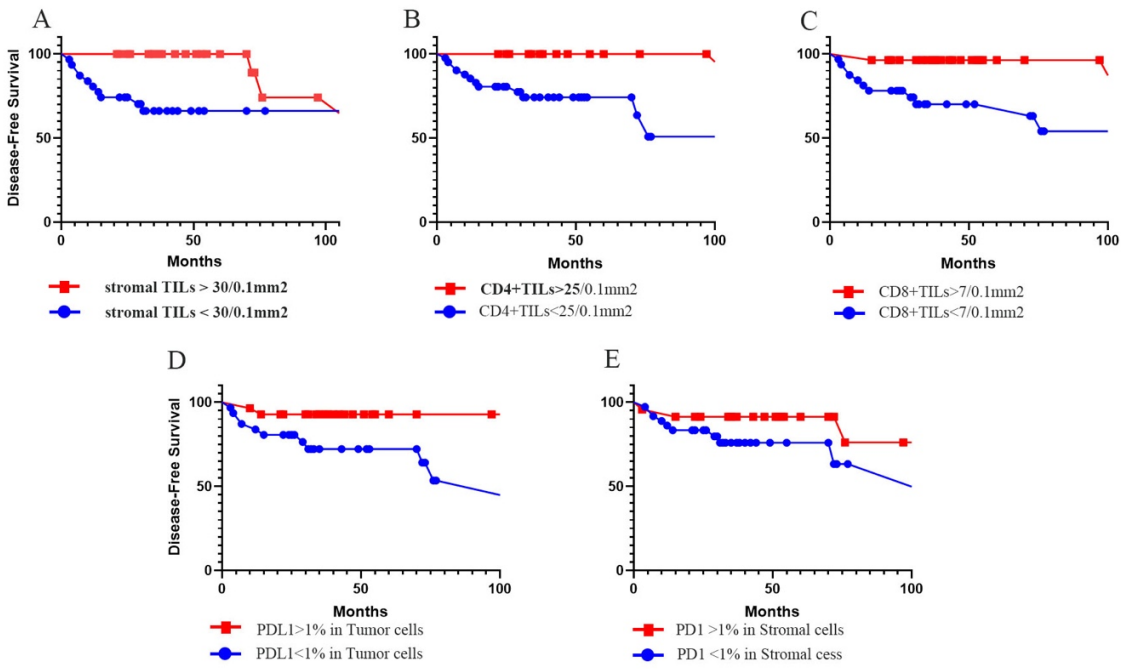

Figure 1 Disease-free survival rates of MBC by stromal TILs (A), CD4+TILs (B), CD8+TILs (C), PDL1 expression in tumor (D), PD1 expression in stroma (E).

Conclusions: These findings support the immune parameters are of prognostic importance in MBC. And provide the clinical significance of applying immune therapies in patients with MBC.

135 Comprehensive Genetic Profiling of Metaplastic Breast Carcinoma with Osseous Differentiation

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Disclosures: Xue Chao: None; Peng Sun: None; Mei Li: None; Rongzhen Luo: None; Rongzhen Luo: None; Jiehua He: None

Background: Metaplastic breast carcinoma with osseous differentiation is a rare histopathological type of breast neoplasms. Clinical outcomes of which were extremely poor and optimal treatment remains challenging. We performed a next generation sequencing to identify genetic alterations of metaplastic breast carcinoma with osseous differentiation, seeking to possible therapy strategies.

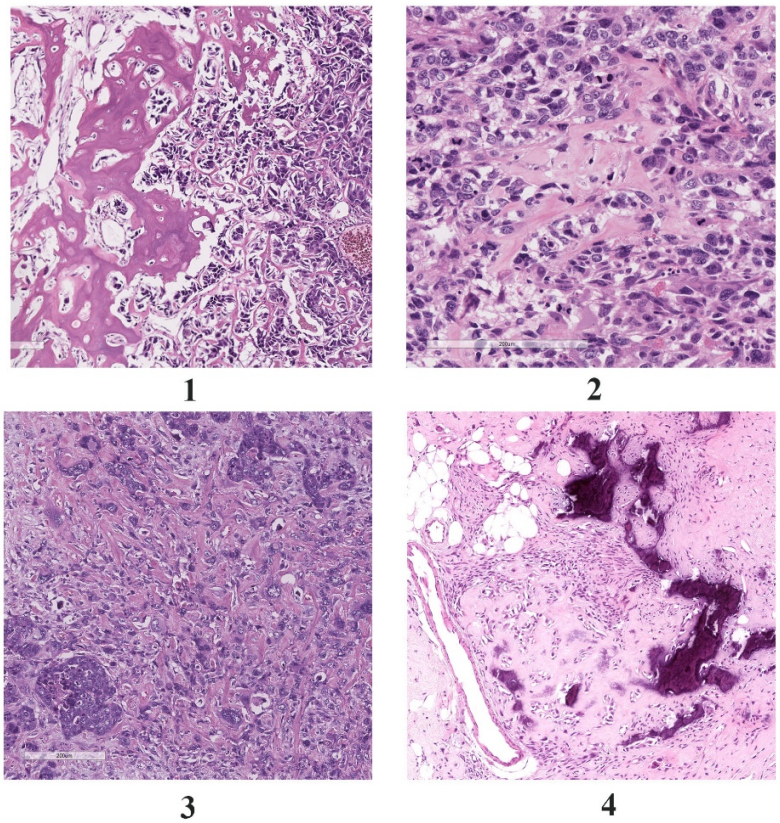
Design: 4 metaplastic breast carcinomas with osseous differentiation without neoadjuvant therapy were included in our study (Figure 1).Comprehensive genomic profiling (CGP) was conducted on DNA/RNA extracted from formalin-fixed paraffin-embedded tissue using the FoundationOne assay. All classes of genetic alterations (GA) including base substitutions, indels, copy number alterations and selected genomic rearrangements (Table 1). Additionally, genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) are reported.

Results: CGP revealed 14 GAs (3.5 per tumor), 3 of which were clinically relevant genomic alterations.3 of 4 patients have detected clinical relevant genomic alterations. Mutations in coding exon 20 (H1047R) of PIK3CA were detected in 2 cases, which indicates that these patients may benefit from mTOR inhibitors, including everolimus and temsirolimus.1 cases detected a classical GA of EGFR (L858R), means the tumor may respond to tyrosine kinase inhibitors. 3/4 patients had TP53 mutations. Other GAs including *RB1*, *TET2*, *PIK3CA*, *FGFR1*, *JUN*, *NSD3, ZNF703*a, *MUTYH* and *RAD21*. All RSs were MSI stable, the mean TMB was 4.75 mutations/megabase (Mb), and none (0%) featured TMB >10 mutations/Mb. Limitations include the small sample size.

Case#	Age	Primary location	Tumor size (cm)	Surgery	Genetic Alterations	TMB burden (/Mb)	MSI-status	Outcome
1	46	right	4	Mastectomy	6,	9Muts	stable	Axillary Metastasis 3 months
2	49	right	2.1	Mastectomy	4	4Muts	stable	Alive 11 months
3	44	left	3.5	Subcutaneous mastectomy+ silicone implataion	3	3Muts	Stable	Lung Metastasis 19 months Brain Metastasis 27 months
4	43	Right	3	Mastectomy	1	3Muts	Stable	Alive 26 months

Figure 1 - 135

Figure 1



Hematoxylin and eosin–stained section of 4 included cases.

Conclusions: PIK3CA and TP53 change were frequently found in metaplastic breast carcinoma with osseous differentiation. All MSI-status of these four cases were stable. 1 case exhibited a TMB burden of 9 mutations/Mb, indicating it may respond to immune therapy. Further study involving epigenetic changes of metaplastic breast carcinoma is awaiting to find possible treatment strategies.

136 High Grade Metaplastic Breast Carcinomas: Better Than Expected Survival Rates in this Subgroup of TILs Poor Triple-Negative Breast Carcinomas

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Disclosures: Suzanne Chartier: None; Caterina Marchio: *Consultant*, Bayer; *Consultant*, Roche; *Consultant*, Daiichi Sankyo; *Consultant*, MSD; Laetitia Fuhrmann: None; Anne Vincent-Salomon: None

Background: High grade metaplastic breast carcinoma (MBC) is a rare subtype of invasive breast carcinoma, mostly triple-negative. It is currently known as being associated with a worse outcome and less sensitivity to chemotherapy than invasive carcinomas of no special type. The goal of this study was to review the clinicopathological features of patients with MBC.

Design: 65 patients diagnosed from 2005 to 2017 in our institution with high grade MBC were retrospectively included. Clinicopathological and immunohistochemical data were reviewed (antibody panel: ER, PR, AR, HER2, Ki67, CK5, CK14, EGFR, SOX10, PDL1 and TROP2).

Results: Median age was 59.5 years (median follow-up: 52 months). 9% of patients had metastatic disease at diagnosis. Nineteen patients received neoadjuvant therapy with 5 pathological complete responses (26% pCR). Most tumors were pT1/pT2 (77%) and 12% were pN+. Histological subtypes (squamous, spindle cells, mesenchymal, mixed) were 35.5%, 9%, 15.5% and 40%, respectively. TILS levels were low (median: 10%) except when squamous differentiation was present. Most of the tumors were triple negative (92%) and expressed at least one of the three surrogate markers (CK5, CK14 or EGFR) for basal-like breast cancer (93%). RA was positive in 19% of the cases, TROP2 85% and SOX10 32%. PDL1 was positive in tumor cells in 18% (cut-off: 1% of positive tumor cells) of the cases and in tumor-infiltrating immune cells in 40% (cut-off: 1% of tumor area). None of the markers studied were specific to one of the subtypes of MBC but SOX10 was negative in pure spindle cell carcinoma and more frequently expressed in pure mesenchymal MBC (7/9 (78%)) than in other types of MBC (12/50 (24%)) ($p<0.05$). Five years survival rates were 88% (overall survival, OS) and 92% (breast cancer specific survival, BCSS). pN+ and lymphovascular invasion were statistically associated ($p < 0.05$) with poorer OS and BCSS. Seven (11%) patients had a local recurrence. Patients with pure spindle cell MBC had statistically worse local recurrence free survival.

Conclusions: Conclusion: MBC demonstrate low TILs breast carcinomas than IC-NST but better survival rates than those reported in the literature. Interestingly, SOX10 was negative in spindle MBC and expressed in the majority of mesenchymal MBC. In a non-negligible proportion of cases, PDL1 and AR expression underpin patients that may benefit from new targeted therapeutic strategies.

137 Mammary Amyloidosis: An Uncommon Entity That May Present as Mammographic Microcalcifications

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Disclosures: Esther Cheng: None; Jessica Chapman-Lim: None; Anna Liu: None; Ahmet Dogan: *Consultant*, Roche, Corvus Pharmaceuticals, Seattle Genetics, Oncology Specialty Group, Pharmacyclics, Celgene, Novartis, Takeda; *Primary Investigator*, ROCHE/GENENTECH; Pedram Argani: None; Timothy D'Alfonso: None; Syed Hoda: None

Background: Amyloidosis of the breast is rare and can occur as a localized process or may be part of a systemic disease including light chain (AL) amyloidosis or amyloidosis associated with chronic inflammatory diseases. The most common clinical presentation is of a palpable unilateral mass that may be associated with calcifications. We set out to further characterize mammary amyloidosis with respect to clinicopathologic features and classification of amyloid precursor proteins.

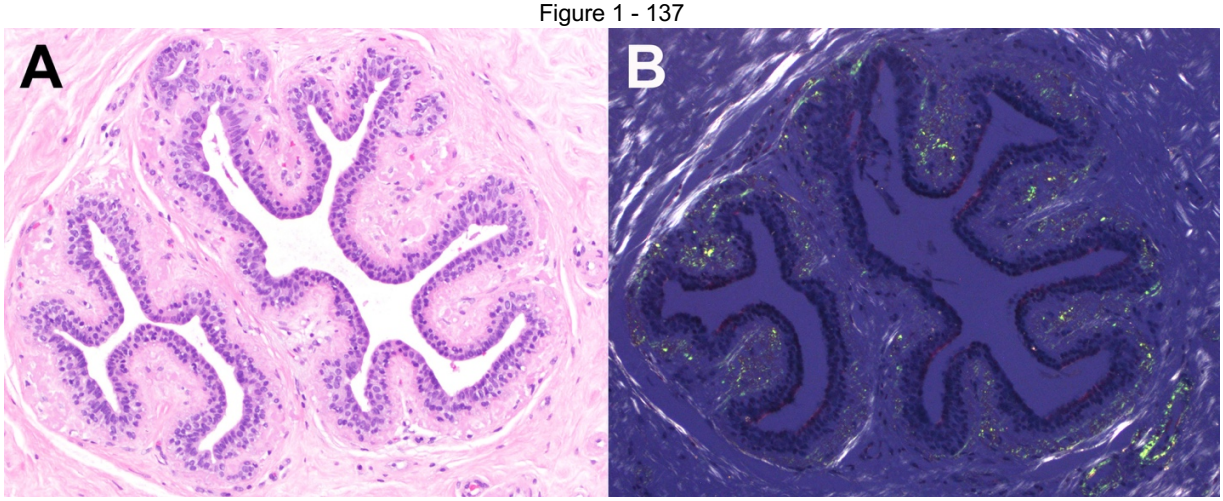
Design: An electronic search was performed over a 20-year period (1/2000 – 1/2019) for mammary amyloidosis from two institutions. H&E and Congo Red slides were reviewed. Clinical and imaging data were retrieved from electronic medical records. Liquid chromatography mass spectrometry (LCMS) was performed on paraffin-embedded tissue blocks in selected cases.

Results: Nine female patients with mammary amyloidosis were identified with a mean age of 65 years (range: 41-84) (Table). Five patients (59%) presented with suspicious mammographic calcifications, one of which was bilateral. One patient presented with bilateral palpable masses. One patient presented with a palpable mass that was found to be invasive mammary carcinoma with adjacent incidental amyloid. The presentations of the remaining 2 patients were unknown. Amyloid deposition was found in stroma, periductal (Figure, A), and perivascular locations. Calcifications typically occurred in stromal amyloid. Congo Red confirmed the presence of amyloid in 8/8 cases tested showing characteristic apple green birefringence (Figure, B). Seven cases submitted for LCMS showed amyloid to be of the AL type in 71% (5/7), of which three patients had a history of hematologic disorders. Two patients had ATTR type precursor protein, both of whom had primary cardiac amyloidosis.

Case	Age (years)	Systemic disease	Presentation	Laterality	Congo Red	LCMS Result
1	59	None	Mammographic calcifications	Left	+	AL, possibly lambda
2	84	Primary cardiac amyloidosis	Mammographic calcifications	Left	+	ATTR
3	78	Multiple myeloma	Mammographic calcifications	Bilateral	+	AL lambda
4	53	None	Mammographic calcifications	Right	Not performed	AL lambda
5	71	None	Mammographic calcifications	Right	+	Not performed
6	41	Sjogren's syndrome	Palpable masses	Bilateral	+	AL lambda

7	77	MGUS/ primary cardiac amyloidosis	Mass (confirmed to be invasive breast carcinoma)	Left	+	ATTR
8	71	None	Unknown	Left	+	AL/AH lambda
9	55	None	Unknown	Left	+	Not performed

Figure 1 - 137



Conclusions: Mammary amyloidosis is an uncommon finding that can present as mammographic calcifications, as a palpable mass, or as an incidental finding. Identification of amyloid in the breast warrants further workup as most cases are associated with systemic diseases.

138 Analysis of Circulating Tumor DNA (ctDNA) in Triple Negative and HER2 Positive Breast Cancer Patients throughout Neoadjuvant Chemotherapy. A Proof of Concept Study

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Disclosures: Nikaoly Ciriaco: None; Esther Zamora: *Speaker*, Novartis; Santiago Escrivá de Romaní: *Advisory Board Member*, Roche; *Speaker*, Roche; *Speaker*, EISAI; Rosa Somoza: None; Javier Hernandez-Losa: None; Santiago Ramon Y Cajal: None; Vicente Peg: *Speaker*, Roche; *Consultant*, Sysmex; Martin Espinosa-Bravo: None

Background: Circulating free DNA was first described over 60 years ago. Since then, its potential use to help clinical management of patients with breast cancer (BC) has been proven. In patients treated with neoadjuvant chemotherapy (NAC), analysis of circulating tumor DNA (ctDNA) has been shown to be useful for the monitoring of minimal residual disease. In the present work we study the variation of ctDNA throughout the neoadjuvant treatment as well as its correlation with the pathological response in the resected specimen and its potential ability to predict pathological complete response.

Design: 18 patients with triple negative (TN) or HER2 positive BC who underwent NAC were analyzed in order to evaluate TP53 (exons 5-9) and PI3KCA (exons 9 and 20) mutations by sanger sequencing in primary FFPE samples. Plasma samples at diagnosis, 1st cycle, middle treatment and at surgery where obtained. For each patient, a mutation in PIK3CA or TP53 identified in the tumor tissue was assessed in the plasma at multiple time points throughout neoadjuvant treatment, using Sysmex Inostics' SafeSEQ technology. SafeSEQ is a next-generation sequencing method which employs Safe-SeqS target enrichment technology (Kinde et al. 2011) and unique molecular identifiers to provide ultra-high sensitivity detection of low frequency mutations present in circulating tumor DNA with maximum specificity. All patients underwent a surgical excision after NAC.

Results: 6 patients (33%) showed TP53 and 2 (11%) PI3KCA mutations in the primary tumor. Both PI3KCA mutations were observed in HER2 positive tumors while TP53 mutations were mainly observed in TNBC (88%). After the 1st cycle of NAC, no mutations were detected

in 6 (75%) patients while the 2 remaining (25%) were no further detected since the middle of the treatment. Interestingly, one patient showed a TP53 baseline mutation (TP53 p.R273L) that was no longer detected after the 1st cycle of NAC but a new mutation was revealed at the time of surgery (TP53 p.R267W), even there was no evidence of malignancy in the resected specimen. 7 (88%) patients reached a pathological complete response (pCR).

Conclusions: ctDNA detected in plasma of patients with TN or HER2 positive BC showed a sharp decrease from the first cycle of NAC reaching undetectable levels until the time of surgery, correlating with pCR. However, new mutations not previously present appeared, even if no residual disease was observed in the surgical specimen, reflecting the adaptation of the tumor to treatment.

139 Regional Lymph Node Status following Neoadjuvant Chemotherapy for Breast Carcinoma - Correlation with Breast Response and Accuracy of Radioactive Seed Localization

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Disclosures: Beth Clark: None; Ronald Johnson: None; Rohit Bhargava: None

Background: Pathologic complete response (pCR) to neoadjuvant chemotherapy (NAT) has prognostic implications, and data on correlation of response between breast and regional lymph nodes (RLNs) is limited. Documentation of retrieval of a previously biopsied positive RLN is important for assessment of response and further management. This may be accomplished through biopsy clip placement at time of biopsy followed by radioactive seed-localization (RSL) prior to definitive surgery. Pathologists document retrieval of biopsy clip, radioactive seed, and histologic biopsy site changes. This study explores the correlation between chemotherapy response in breast and RLNs and evaluates the accuracy of RSL of RLNs.

Design: Copath Natural Language search was used to identify 155 post-neoadjuvant surgical specimens from 153 patients. Cases were segregated based on pre-therapy lymph node status. Pre-and post-therapy pathology data, including tumor type, biomarker status, response to therapy, evidence of biopsy site changes, and clip retrieval were recorded. Fisher's exact test was used to evaluate impact of hormone receptor (HR) and HER2 status.

Results: In 109 cases, pre-NAT biopsy of RLN was performed: 106 core biopsy and 3 fine needle aspiration biopsy (FNA). Following NAT, RSL of RLN was performed in 63/74 cases with positive RLN biopsy, 7/35(20%) cases with negative RLN biopsy, and 0/46 cases with no pre-NAT RLN biopsy. Overall pCR rate was 33%. Breast and RLN correlation and post-NAT RLN evaluation results shown in Table 1. HR and HER2 status did not impact correlation rates (Figure 1). Retrieval of biopsied RLN in 6/74 cases could not be confirmed due to: RSL failure (1), clip placement failure (1), possible insufficient pathology evaluation (1), no biopsy clip placed (2), indeterminate (1).

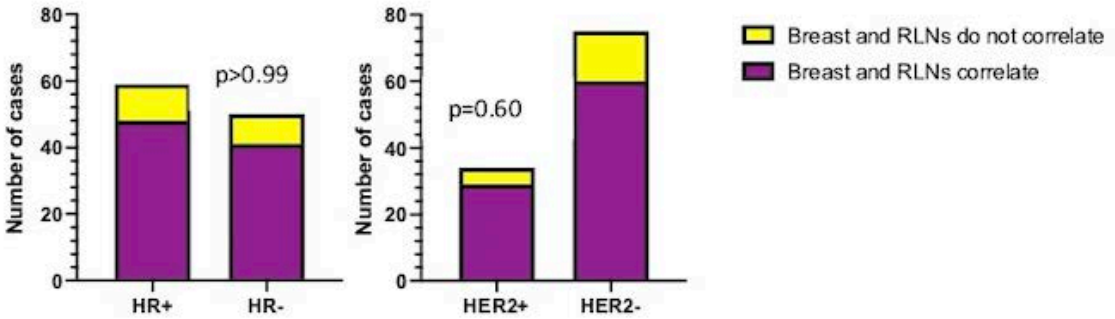
Six of 35 (17%) cases with negative pre-therapy RLN biopsy had residual RLN involvement, and 7/46 (15%) cases without pre-therapy RLN biopsy had residual RLN involvement, with all cases also showing residual disease in the breast.

Table 1: Post Neoadjuvant Regional Lymph Node (RLN) Evaluation in 109 cases with Pre-Therapy Biopsy

	Pre-Therapy RLN biopsy positive (n=74)	Pre-Therapy RLN biopsy negative (n=35)
Agreement of response between breast and RLNs	63 (85%)	25 (71%)
Residual Disease in Breast Only	8 (11%)	10 (29%)
Residual Disease in RLNs Only	3 (4%)	0 (0%)
Radioactive seed localization performed	63 (85%)	7 (20%)
Axillary Lymph Node Dissection	11 (15%)	3 (9%)
Retrieval of Previously biopsied RLN confirmed	67 (92%)	26 (74%)

Figure 1 - 139

Figure 1: Correlation of Breast and Regional Lymph Node (RLN) Response by Biomarker Phenotype in 109 cases with pre-therapy RLN biopsy by Hormone Receptor (HR) and HER2 Status



Conclusions: Correlation between breast and RLN response to neoadjuvant chemotherapy is high, with < 2% cases overall showing complete response in the breast but residual RLN disease. Pathology documentation of previously biopsied RLN is important for accurate staging and determination of response to NAT. Additional patient or specimen imaging, deeper H&E levels, and radiology-pathology correlation may be needed when retrieval of previously involved RLN cannot be confirmed.

140 Identification of Patients with Atypical Ductal Hyperplasia (ADH) on Core Needle Biopsy (CNB) at High and Low Risk of Upgrade at Surgical Excision: A Contemporary Series of 286 Cases

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Disclosures: Allison Cleary: None; Samantha Grossmith: None; Claire King: None; Maya Harary: None; Beth Harrison: None; Elizabeth Mittendorf: None; Stuart Schnitt: None; Tari King: None

Background: 15-25% of patients diagnosed with ADH on CNB will have an upgrade to ductal carcinoma in situ (DCIS) or invasive breast cancer (IBC) at surgical excision (SE). Reliable identification of patients at low risk for upgrade who can be spared SE remains an important clinical goal.

Design: 427 patients with ADH on CNB who underwent subsequent SE from Jan 2000- March 2018 were identified. After excluding patients for whom slides were not available, those with a prior or current history of ipsilateral breast cancer, those in which the ADH involved another lesion, and MRI biopsies there were 286 patients available for analysis. Clinical and radiographic features and histologic sections of the CNB were reviewed blinded to outcome.

Results: CNB was performed for microcalcifications in 91%; 8 - 11G needles and vacuum assistance were used in 93% of cases. 46 patients (16%) had an upgrade to DCIS (n= 39; 14%) or IBC (n=7; 2%) at SE. Upgrade was not significantly related to any clinical features (age, race, menopausal status, family history of breast cancer, Gail risk score). There was no association between risk of upgrade and the nature of the imaging target, imaging guidance method, or needle gauge. Pathologic features significantly associated with upgrade were the number of terminal duct lobular units (TDLUs) involved by ADH (mean 4.6 in patients with upgrade vs 2.7 in patients without upgrade; p= 0.0001) and severity of atypia (upgrade rate 33% for cases categorized as severely atypical bordering on DCIS vs 7% for other cases; p=0.0001). Among 24 cases with both $\geq$3 involved TDLUs and severe atypia bordering on DCIS, the upgrade rate was 54%. Conversely, among the 150 cases with $\leq$2 involved TDLUs and without severe atypia bordering on DCIS, the upgrade rate was 10%. Among 83 cases where ADH involved only 1 TDLU without severe atypia bordering on DCIS, the upgrade rate was 7.2 % (5 cases of DCIS and 1 case of DCIS + IBC).

Conclusions: To our knowledge, this is the largest study of ADH on CNB with central pathology review of all cases. Our results indicate that a more limited extent of ADH and absence of severe atypia bordering on DCIS were associated with lower upgrade rates. However, the upgrade rate among this low risk group is still ~7%. We are currently evaluating whether a machine learning model combining clinical, imaging and pathologic features can improve the identification of patients with ADH on CNB who can be spared surgical excision.

141 Clinical and Pathologic Characterization of Secondary Inflammatory Breast Cancer

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Disclosures: Allison Cleary: None; Beth Harrison: None; Marie Claire Remolano: None; Faina Nakhlis: None; Jennifer Bellon: None; Heather Jacene: *Advisory Board Member*, Advanced Accelerator Applications USA, Inc; *Speaker*, Janssen Pharmaceuticals; *Grant or Research Support*, Siemens Healthcare, Inc; *Grant or Research Support*, GTx, Inc; Eren Yeh: None; Caroline Block: None; Beth Overmoyer: None

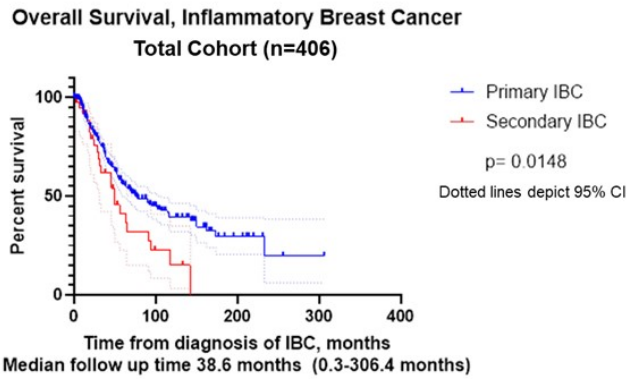
Background: Inflammatory breast cancer (IBC) is a rare and aggressive subtype of breast cancer that poses significant diagnostic and treatment challenges. Particularly little is known about patients with a secondary IBC arising in a breast previously treated for non-inflammatory breast cancer. In this study, we sought to characterize the clinical and pathologic features of secondary IBC compared with de novo or primary IBC.

Design: Retrospective analysis of clinical and pathologic data was performed for a cohort of 406 patients with clinically confirmed IBC who were enrolled on an IRB approved IBC registry between July 1991 and Oct 2018.

Results: Of the 406 IBC pts in this cohort, 38 (9.4%) had a personal history of prior ipsilateral non-inflammatory breast cancer, with a median interval of 108 months (mo) between the diagnosis of historical breast cancer and the secondary IBC (range 6-297 mo). 80% (28/35) of the historical breast cancers were invasive carcinomas; the majority (22/28=79%) being invasive ductal carcinomas (IDC). Most (15/23=65%) were poorly differentiated, and either estrogen receptor (ER)-positive/HER2-negative (15/31=48%) or triple negative (TN, 9/29=31%). Similarly, the majority of tumors in both the secondary IBC and primary IBC groups were poorly differentiated IDC (26/37=70% vs 229/355=65%). However, when secondary IBC was compared with primary IBC, there was a significant difference in receptor profiles (p=0.009462): secondary IBC had a higher proportion of TN disease compared with primary IBC (14/36=39% vs 92/359=26%), while primary IBC had a higher proportion of HER2-positive disease (151/353=43% vs 5/39=13%).

Secondary IBC tended to arise in older women compared with primary IBC (50.8 vs 60.6 yrs p=0.0001) . Both secondary and primary IBC pts had similar rates of de novo metastatic disease (12/36=33% vs 107/361=30%). While outcomes were generally poor for both groups, there was a striking decrease in overall survival (OS) for secondary IBC compared with primary IBC (median OS=75 mo vs 49 mo, p=0.0148).

Figure 1 - 141



Conclusions: Secondary IBC demonstrates distinct clinical and pathologic features and follows a more aggressive clinical course compared with primary IBC. These results shed light on a very rare, previously poorly characterized, subset of breast cancer that warrants increased investigation.

142 WNT/β-Catenin Signaling Correlates with Improved Survival in Luminal A Breast Cancer

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Disclosures: Kimberly Cole: None; Kerry Councilman: None

Background: There is evidence implicating WNT/β-catenin signaling in the pathogenesis of breast cancer. IHC studies have demonstrated nuclear expression of β-catenin in tumor samples, indicating activation of the pathway, while normal samples showed a membranous, or inactive, staining pattern. Elevated protein levels of β-catenin have also been detected by Western blotting of breast tumor lysates. Additionally, a correlation was detected between beta-catenin and cyclinD1 (known oncogene) activation. Therefore, it is currently accepted that activation of the canonical WNT signaling pathway contributes to tumorigenesis in many breast cancers. Most of the studies thus far have focused on its role as a predictor of poor outcome in Basal-like cancers. Here we look at the potential role of WNT/β-catenin signaling as a predictive marker in Luminal A breast cancers.

Design: Using reverse phase protein array (RPPA) data from the TCGA breast invasive carcinoma dataset (USCS Cancer Genome Browser), primary tumor samples that had PAM50 subtyping were isolated. Beta-catenin protein levels were examined and compared across the dataset by PAM50 subtype and compared with other protein values.

Survival curve analyses were performed using the KMPlotter software based on microarray-based RNA expression data for the indicated genes.

Results: A TCGA analysis (Zhang, Borcherding et al, 2016) showed that protein expression correlating with β-catenin differs in Luminal A vs. Basal-like cancers. In Luminal A breast cancers, β-catenin expression is associated with ER pathway proteins. In basal-like cancers, it is associated with WNT signaling pathway proteins (Fig 1).

Here we found that improved overall survival in Luminal A breast cancers was significantly correlated with high RNA expression of multiple WNT/β-catenin pathway markers (β-catenin, LEF1, TCF4, FZD1, WNT1 and DVL3). For GSK3B, which is part of the inhibitory complex that blocks WNT signaling, RNA levels did not correlate with survival (Fig 2).

When a correlation was observed in Luminal B and Basal-like cancers for any of the genes, expression correlated both with improved and decreased survival, depending on the specific gene (Fig 2).

Figure 1 - 142

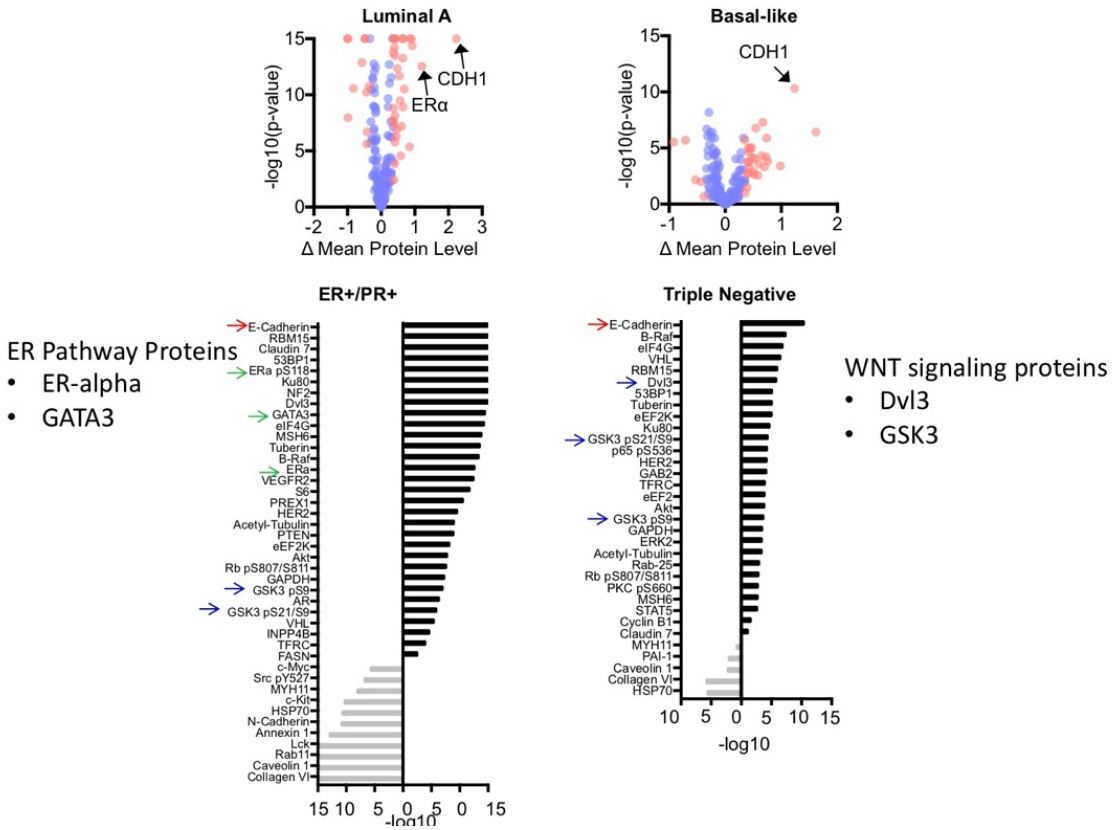
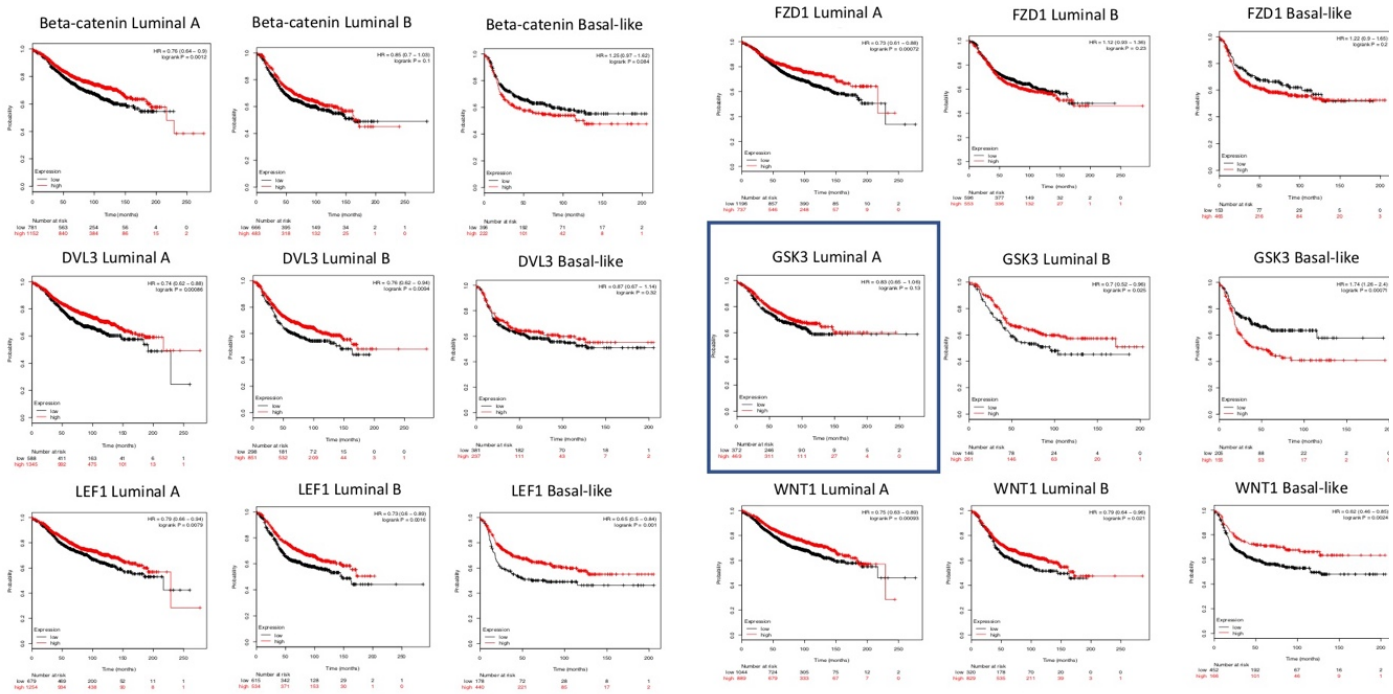


Figure 2 - 142



Conclusions: Activation of WNT/β-catenin signaling is associated with improved survival in Luminal A breast cancers. This supports prior evidence that WNT/β-catenin signaling activates the ER pathway in ER positive cancers.

143 Coding-Free Platform for Development of Image Analysis Algorithm Produces Comparable Results to Established Convoluted Neural Network Architecture for Sub-Classifications of Ductal Carcinoma in Situ of the Breast

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Disclosures: Lorraine Colon Cartagena: None; Matthew Gayhart: None; Valentina Robila: None

Background: Deep learning is an essential component in advancing the efforts of digital pathology to improve diagnostic accuracy and patient care. It can encompass algorithms that can be trained and tested for image classification and diagnostic purposes. Although many breakthroughs in pathology have been developed through this technology, deep learning algorithms can be complex to generate and difficult to interpret. Here, we demonstrate the utility and validation of a coding-free deep learning image classifier using histologic variants of high grade ductal carcinoma in situ (DCIS) of the breast, which represent a well classified and commonly encountered breast pathology entity suitable for validation and adjunct classification by machine learning algorithms.

Design: A total of 334 high-resolution images of variants of DCIS (comedo-type: 97, cribriform: 72, micropapillary: 69, and solid: 96) were obtained from our departmental archives. The images were used to train two deep learning algorithms including the well-studied ResNet-50 model and a coding-free custom image classifier and visual recognition model created with the IBM Watson Visual Recognition platform. A total of 80 high resolution images from our institutional archives were used to test both algorithms (Figure 1). 31 images from outside cases were used to assess the accuracy of the coding-free image classifier, to test for generalizability.

Results: Our results highlight that the ResNet-50 model and the coding-free image classifier have a comparable accuracy of 88% and 90%, respectively (Table 1). Both models predicted correct outcomes with an average confidence score of > 0.80. Outside image cases were correctly classified by the coding-free image classifier with an accuracy of 90%, thus highlighting its generalizability.

Table 1. Test results of image classifying models for variants of DCIS		
DCIS histological subtype	*ResNet-50 Model* *Accuracy/No of cases*	*IBM Watson Visual Recognition Model* *Accuracy/No of cases*
Comedo-type DCIS	89% (17/19)	90% (18/20)
Cribriform DCIS	90% (19/21)	80% (16/20)
Micropapillary DCIS	94% (17/18)	90% (18/20)
Solid DCIS	77% (17/22)	100% (20/20)
Overall accuracy	88% (70/80)	90% (72/80)

Figure 1 - 143

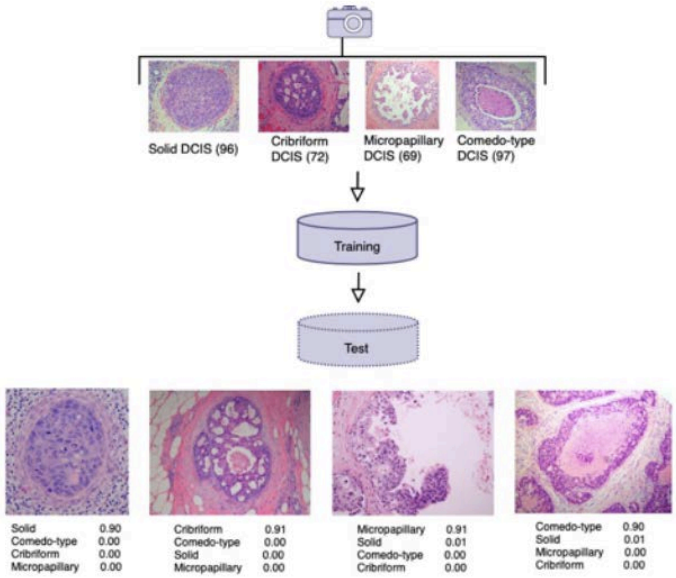

Figure 1. Workflow model for training and testing both image classifying algorithms for variants of DCIS

Conclusions: Our results validate the use of a generalizable coding-free custom image classifier and visual recognition model in the classification of variants of DCIS of the breast. We highlight this tool as an alternative to train a custom image classification model without having to embark into coding architecturally complex deep learning algorithms.

144 Classification of Fibroepithelial Lesions of the Breast Using Deep Learning Models: A Pilot Study

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Disclosures: Lorraine Colon Cartagena: None; Matthew Gayhart: None; Valentina Robila: None

Background: Fibroepithelial lesions (FELs) of the breast include a spectrum of neoplasms ranging from fibroadenomas (FAs) and variants of phyllodes tumors (PTs). It is well known that the classification of FELs can represent a challenge in core needle biopsies (CNBs), when overlapping features between these lesions may be present. In those instances, a diagnosis of FEL is often rendered thus leading to surgical excision for definitive characterization. Here, we demonstrate how the use of two custom deep learning and visual recognition models aids in the classification of challenging FELs in CNBs of the breast.

Design: 92 cases diagnosed as FEL on CNB, but with a corroborating diagnosis of FA or benign PT on excision were retrieved from our departmental archives. 590 high resolution images were obtained from whole slide imaging of the CNBs (FA: 373, PT: 217). The well-studied deep learning ResNet-50 model was trained using 472 images, and then tested for classification of 118 images (FA: 68, PT: 50). We also used a coding-free custom image classifier and visual recognition model created with the IBM Watson Visual Recognition platform, that was trained with 437 images and tested for an additional 153 images (FA: 81, PT: 72).

Results: The average confidence score for both models and FA and PT categories was of > 0.8. The coding-free image classifier showed an overall increased accuracy, failing to correctly predict 7% of the images compared with 17% by the ResNet-50 model (Table 1). Both

models performed comparably for FA identification. However, the coding-free image classifier showed greater specificity than the ResNet-50 for PT classification.

Table 1. Test results of image classifying models for FELs

	ResNet-50 Model	IBM Watson Visual Recognition Model
Fibroadenoma	89.7% (61/68)	87.7% (71/81)
Phyllodes tumor	74.0% (37/50)	98.6% (71/72)
Overall	83.1% (98/118)	92.8% (142/153)

Conclusions: Both visual recognition models tested show high accuracy in the classification of challenging FELs of the breast. In our cohort, the coding-free image classification model has increased specificity for PTs when compared with the ResNet-50. The results showcase that the coding-free model is an accessible, easy to interpret tool that shows a comparable performance to the established ResNet-50 model and highlight the importance of incorporating deep learning in our pathology practice as an adjunct tool for the evaluation of complex cases.

145 Clinico-Pathological and Molecular Portrait of Breast Carcinomas with Osteoclast-Like Giant Cells

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Disclosures: Joanna Cyrta: None; Laetitia Fuhrmann: None; Odette Mariani: None; Suzanne Chartier: None; Andre Vieira: None; Anne Vincent-Salomon: None

Background: Breast carcinomas (Ca) with osteoclast-like giant cells (COGC) are very rare. They show distinct stromal features, but their molecular characteristics remain insufficiently known. We report clinical and pathology data for 16 CCGO, and whole exome sequencing (WES) and RNA-seq results for a subset of cases.

Design: All cases diagnosed at our institution in 2000-2019 were included. Clinical and pathology data were retrospectively reviewed. IHC for CD34, CD68, CD163 and Perls' stain were done on 11 COGC and 10 matched non-COCG Ca. WES (8 COGC tumor/normal pairs) and RNA-seq (7 COGC and 7 matched non-COCG Ca) were performed on nucleic acids extracted from fresh frozen tissue (NovaSeq6000, PE100).

Results: Histology subtypes of COGC were: 11 (69%) non-specific type Ca (NST), four (25%) metaplastic Ca (MC) and one mixed NST/lobular Ca with focal COGC. Median age at diagnosis (years) was 46 (33-68) for COGC-NST and 72.5 (53-84) for COGC-MC. Median tumor size was 18mm (6-32) for COCG-NST and 30mm (21-35) for COCG-MC. Elston-Ellis grade was 1 (n=4), 2 (n=6) or 3 (n=1) for COCG-NST and 3 for all COCG-MC. All COGC-NST were ER+, PR+, HER2-, Ki67 was <14% in 5 (45%) cases. All COGC-MC were triple-negative with Ki67>20%. Two COGC-NST and the mixed case had axillary lymph node metastases. All COGC-TNS patients were alive disease-free at median follow-up of 71 months (21-128) for COCG-TNS and one COCG-MC patient had inoperable recurrence, median follow-up 27 months (10-74). The patient with mixed Ca died of disease (metastases were non-COGC).

There was evidence of recent or past stromal hemorrhage in all COGC cases and only one control. CD68+ infiltrate and CD34+ vessels were significantly more abundant in COGC than in controls, while CD163+ infiltrate and tumor-infiltrating lymphocytes were not. WES revealed mutations in *PIK3CA* (n=2), *MAP3K1* (n=1), *MAP2K4* (n=1), *AKT1* (n=1) and *BRAF* (n=1), but not *TP53*. Most cases (n=6) had simplex genomic profiles; 11p gain/16q loss (n=2), 8p loss (n=2), 16q loss (n=2) were seen.

Transcriptomic profiles of COGC differed strikingly from controls. Upregulated pathways included osteoclast maturation and function. There was significant upregulation of *RANK, RANKL* and downregulation of *OPG.*

Conclusions: Most COCG are luminal A or B IC-NST in young patients, and a subset are MC. Their genomics are somewhat similar to non-COCG luminal IC-NST except for absence of *TP53* mutations, but gene expression profiles are strikingly different. Exploring the RANK/RANKL/OPG axis appears warranted.

146 Breast Sentinel Lymph Node Frozen Section Practice: An Enterprise Audit as a Guide for Moving Forward

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Disclosures: Rebecca Czaja: None; Julie Jorns: None

Background: It is standard of care to process sentinel lymph nodes (SLN) by slicing at 2 mm intervals, with the goal of detecting macrometastatic disease (≥2 mm). CAP protocols outline that at least one H&E level per SLN be evaluated, with one level deemed adequate, given appropriate sectioning, in recent publications. However, we, like many other practices perform multiple levels and/or immunohistochemistry (IHC). We sought to evaluate our practice and outcomes, with focus on more challenging SLN frozen section (FS) cases, in consideration of institutional protocol changes.

Design: We conducted a retrospective database search (8/17-7/18) for cases of breast SLN FS from our enterprise (1 academic institution (AI) and 2 community sites (CS)). Clinicopathological features were assessed by chart and slide review. Cases were reviewed for grossing technique and discordance between FS and permanent section (PS) (i.e. false negatives (FN) due to sampling (SE) or interpretive error (IE)).

Results: Of 216 patients, 99.1% were female, avg 57.2 yrs, with 29.6% having undergone neoadjuvant chemotherapy. (Table 1) 189 (87.5%) cases were from the AI and 27 (12.5%) from CS.

Adherence to the 2 mm sectioning protocol was higher at the AI (157/189, 83.1%) vs CS (4/27, 12.5%) , where SLN were more frequently bisected, regardless of size.

11/216 (5.1%) had FN FS results, with 4 (1.85%) having micrometastatic, 3 (1.4%) pN1 and 4 (1.85%) pN2 disease, with median metastatic size of 0.2 cm (range 0.1-0.9 cm). FN were due to SE for 9 (81.8%) and IE for 2 (18.2%). Two patients (0.9%) with FN FS later went axillary lymph node dissection (ALND). Most (8/9, 88.9%) with FN FS due to SE had metastasis identified on the 1st PS level (of 3) and the other was identified on the 2nd PS level.

Higher FN was seen at CS (2/27, 7.4%) vs AI (9/189, 4.8%).

Of 7 patients with FN FS and macrometastatic disease, 3 (42.9%) were not serially sectioned; the other 4 (57.1%) underwent neoadjuvant chemotherapy. (Figure 1) (Figure 2)

Table 1: Clinicopathological features (N=216).

Sex (N (%))	
Female	214 (99.1)
Male	2 (0.9)
Age (mean (range)) (yrs)	57 (27-90)
Pathologic T (pT) Stage (N (%))	
0/is	53 (24.5)
1	119 (55.1)
2	41 (19.0)
3	1 (0.5)
X[1]	2 (0.9)
Pathologic N (pN) Stage (N (%))	
0	174 (80.6)
1mi	8 (3.7)
1	21 (9.7)
2	10 (4.6)
3	2 (0.9)
X[2]	1 (0.5)
Histology (N (%))	
DCIS/PLIS	37 (17.1)
IDC	144 (66.7)
ILC	28 (13.0)

Other	7 (3.2)
Metastasis (M) Stage (N (%))	
M0	214 (99.1)
M1	2 (0.9)
Neoadjuvant Chemotherapy (N (%))	64 (29.6)
ALND (N (%))	29 (13.4)
Axillary Radiation (N (%))	23 (10.6)

[1] pT stage X was due to the presence of lymphatic involvement only following neoadjuvant chemotherapy. [2]pN stage X was due to the presence of metastases to contralateral axilla SLN only (ipsilateral SLN not assessed); this case represents one of two with M1 status).

Abbreviations: DCIS/PLIS – Ductal carcinoma in situ/pleomorphic lobular carcinoma in situ; IDC - Invasive ductal carcinoma; ILC – Invasive lobular carcinoma; ALND – Axillary lymph node dissection.

Figure 1 - 146

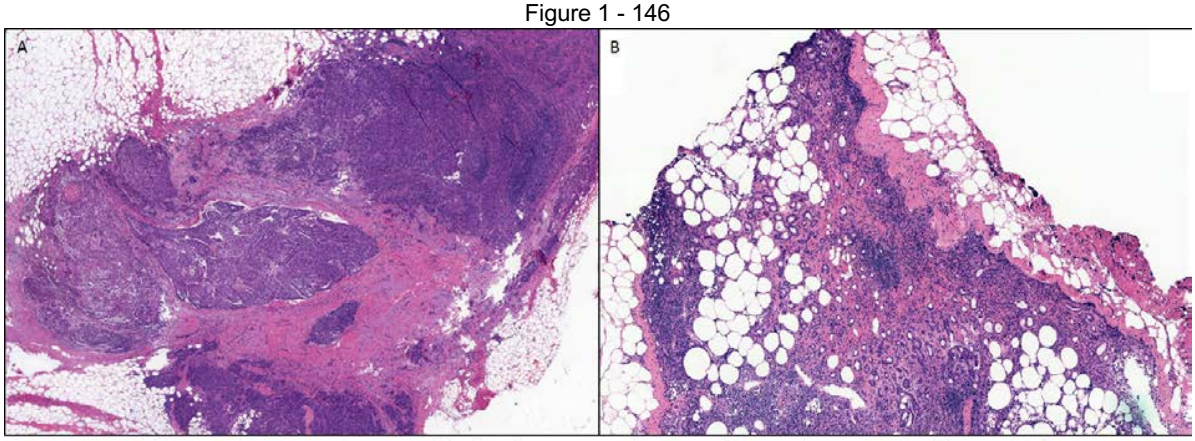

Figure 1. Example false negative frozen section sentinel lymph node cases secondary to sampling error (i.e. metastasis only present in deeper permanent sections): A. Macrometastatic ductal carcinoma in a bisected lymph node and B. Micrometastatic ductal carcinoma in a serially-sectioned lymph node.

Figure 2 - 146

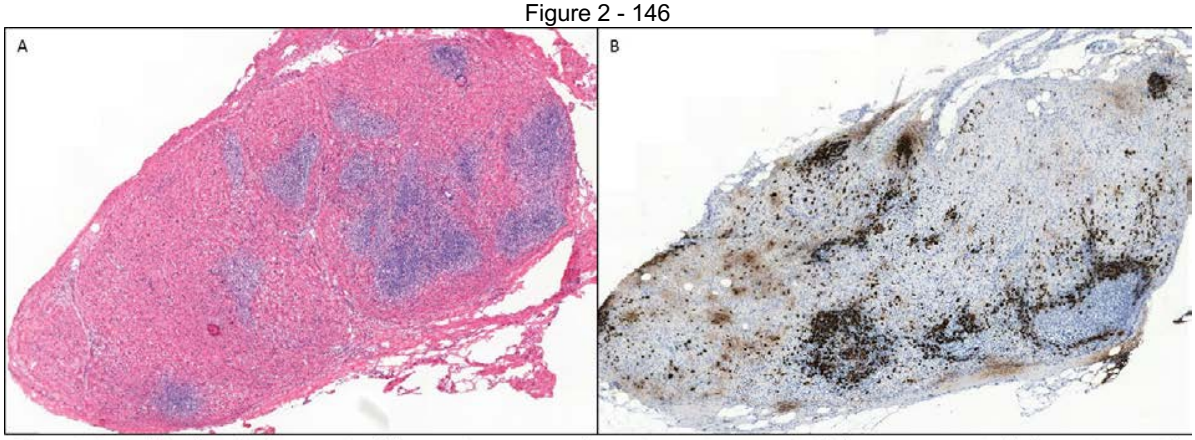

Figure 2. Sentinel lymph node frozen section false negative case secondary to interpretive error: A. Subtle macrometastatic lobular carcinoma with therapy effect on frozen section with B. Metastatic carcinoma highlighted by cytokeratin AE1/AE3 immunohistochemistry.

Conclusions: Review of SLN FS cases highlighted gaps in awareness of institutional protocol (an educational opportunity), especially at CS. Most discordant cases were small metastases, due to SE and did not elicit ALND. Additionally, most FN FS due to SE were identified on the first PS level, supporting decreasing PS levels going forward. We plan to increase the timeframe of this study to further support this practice change.

147 TERT Promoter Mutations in Metaplastic Breast Cancer

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Disclosures: Edaise M. da Silva: None; Mahsa Vahdatinia: None; Fresia Pareja: None; Pier Selenica: None; Felipe Geyer: *Employee*, Novartis; Ferrando Lorenzo: None; Ariya Bakhteri: None; Simon Lee: None; Arnaud Da Cruz Paula: None; Salvatore Piscuoglio: None; Charlotte Ng: None; Hannah Wen: None; Edi Brogi: None; Hong (Amy) Zhang: None; Britta Weigelt: None; Jorge Reis-Filho: None

Background: Metaplastic breast cancer (MBC) is a rare histologic subtype of triple-negative breast cancer. MBCs are characterized by the differentiation neoplastic epithelium into squamous, spindle, chondroid or osseous components. MBCs commonly harbor *TP53* mutations, which are less frequent in MBCs with a predominant spindle cell component. *TERT* promoter mutations, reported in up to 52% of phyllodes tumors of the breast, are thought to be absent or extremely rare in breast carcinomas. Here, we sought to determine the frequency of *TERT* promoter hotspot mutations in MBCs.

Design: Forty-three MBCs were centrally reviewed according to the criteria put forward by the World Health Organization. Estrogen receptor (ER) and HER2 status were defined according to the ASCO/CAP guidelines. All MBCs were subjected to Sanger sequencing for *TERT* promoter hotspot loci. *TP53* mutation status was available in 22/43 cases. In addition, 8 MBCs previously subjected to targeted sequencing were interrogated for *TERT* promoter and *TP53* mutations.

Results: Our series was comprised of 43 MBCs classified into MBCs with predominant chondroid (46%, 20/43), spindle cell (26%; 11/43), osseous (7%; 3/43),) and squamous (21%; 9/43) components. 91% (39/43) and 9% (4/43) of MBCs were of histologic grades 2 and 3, respectively. The vast majority of MBCs (95%; 41/43) were ER- /HER2- and 5% (2/43) were ER- /HER2+. *TERT* promoter C228T mutations were identified in 14% (6/43) of cases interrogated, including MBCs with a predominant spindle (n=3), squamous (n=2) and osseous (n=1) components. Of note, the *TERT* mutated MBC with a predominant osseous component (80%) also exhibited a minor spindle cell component (20%). One case from the additional cohort (11%, 1/8) of MBCs harbored *TERT* C228T promoter mutation. Interestingly, 63% (19/30) of the MBCs analyzed harbored *TP53* mutations; of the 3 cases harboring *TERT* promoter hotspot mutations, 66.7% (2/3) were *TP53* wild-type.

Conclusions: A subset of MBCs harbor *TERT* promoter hotspot mutations and appear to be enriched for tumors with predominant spindle or squamous cell differentiation. *TERT* promoter hotspot mutations appear to predominantly affect *TP53* wild-type MBCs. Our findings suggest that the assessment of *TERT* promoter mutation might not be suitable for the differential diagnosis of spindle cell MBCs and phyllodes tumors.

148 Deep Learning Applied to the Highly Subjective Nuclear Grading of Breast Cancer: Correlation with Patient Outcome

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Disclosures: Leslie Dalton: None

Background: Nuclear grading (NG) of breast cancer has been shown to provide significant ability in risk stratification of patients with invasive breast cancer. But, NG is not regarded as an acceptable biomarker given subjectivity in assessment. The presumed objectivity of deep learning (DL) might provide a remedy, but first a DL method must show ability in predicting patient outcome (PO).

Design: Used were H&E stained tissue microarrays (TMAs) originally prepared by USA National Cancer Institute and currently administered by Univ. Virginia. Obtained were whole slide images of each TMA. The so-called progressive TMAs served for training a model and the test set was comprised of 797 tumors from the prognostic TMAs. To train the model, a single pathologist separated thousands of image panels into high, and low grades as well as stroma. Intermediate grade was assigned by borderline results between the extremes. The algorithm, a convolutional neural network, was coded in python with use of functions from open source keras/tensorflow.

Results: By Kaplan-Meier, deep-learning grade (DLG) corresponded to $p < 0.00001$ across all cancers in patients <=70 yo. P-values were also < 0.00001 by the original full tissue section pleomorphism score (PS) and the grade assessed by examining only the TMA sample (TMA-NG). Time-dependent receiver operator curves revealed area under curves (AUCs): DLG=0.63.8; TMA-NG= 64.9; PS=60.4; and Nottingham score (NS) 65.7. After DLG was substituted for PS in summation of NS, AUC = 67.7. The sum of TMA-NG, PS and DLG corresponded to AUC=66.7. No significant difference was seen in delta AUC between DLG versus PS, or DLG versus TMA-NG.

Conclusions: In the brief space allowed for reporting results, survival statistics were emphasized since patient outcome (PO) is the optimal endpoint to judge merit as compared to a nebulous kappa threshold (and kappa statistic results were not impressive). In predicting PO, DLG was comparable with pathologist assigned grade, but not an improvement. An advantage of DLG, is a model can be validated at level of PO, and then widely deployed. The AUC of the sum of DLG, PS, and TMA-NG was higher than the separate evaluations of each. This would not occur with complete redundancy in evaluations. This suggests that discordance among several opinions added value. At this juncture, DLG shows promise at being a source for an additional opinion. DLG is not ready for daily practice.

149 Organized Lymphoid Structures Stratify Risk in Breast Cancer and Correlate with a Naive B cell Signature

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Disclosures: Hany Deirawan: None; Kristen Purrington: None; Valerie Ratliff: None; Ann Schwartz: None; MHD Fayez Daaboul: None; Saivaishnavi Kamatham: None; Joseph Trak: None; Kang Chen: None; Rouba Ali-Fehmi: None; Sudeshna Bandyopadhyay: None

Background: Despite the advances in the diagnosis and treatment of breast cancer, triple negative breast cancer (TNBC) continues to carry a worse prognosis and disproportionately affects African American women (AAW). Deciphering the immune microenvironment can improve the diagnostic yield of pathological specimens.

Design: We analyzed H&E slides from two different cohorts. The first cohort included women with invasive breast cancer (n=117 black, n=102 white). The second was a cohort of black women with invasive TNBC between 2007 and 2013, who did not undergo neoadjuvant therapy (n=155). Clinical and demographic data in both phases were obtained. Two pathologists blinded to the clinical, molecular, and demographic characteristics associated with the tumors, examined and quantified the extent and pattern of lymphocyte distribution within the tumor and native surrounding tissue. Lymphocyte aggregates configured as clusters of cells surrounding a high endothelial venule or capillary were designated as organized lymphoid structures (OLS). Immune cell subtypes were estimated from expression data of the TNBC cohort using CIBERSORT. Associations were evaluated using logistic regression while survival was evaluated using Cox regression.

Results: Within the first cohort, tumor-infiltrating lymphocytes (TILs) were present in 60.4% of the tumors and women with TNBC were 3.6-fold more likely to have TILs present (p=0.004). Race was not associated with TILs. Tumors with TILs were 4.4-fold more likely to have OLS present (p<0.001), regardless of the percent of TILs. Presence of OLS was independently predicted by both race and subtype, where black women were 2.4-fold more likely (p=0.009) and TNBC were 2.3-fold more likely to have OLS (p=0.035). Given this racial and subtype associations with OLS, we evaluated relationships between OLS, its composition and survival in a cohort of black women with TNBC. OLS were present in 51.3% of tumors, similar to TNBC of black women in the first cohort (58.3%). OLS was not associated with survival in AAW with TNBC when adjusting for TNM stage, age, chemotherapy, and TILs. Immune cell subtypes in OLS were enriched for naïve B cells (p=0.008) and surprisingly a T cell polarization toward follicular helper T cells signature (p=0.003).

Conclusions: OLS can be sites of de-novo adaptive immune response or a mechanism for cancer immune evasion. An integrative histopathological and molecular characterization is a convenient and fast platform for hypothesis generation and validation.

150 Automatic Tumor Nuclei Detection in Core Biopsies by Artificial Intelligence Can Predict Response to Neoadjuvant Chemotherapy in High Risk Breast Cancer Patients

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Disclosures: David Dodington: None; Andrew Lagree: None; Sami Tabbarah: None; Tina Wu: None; Christianne Hoey: None; William Tran: None; Fang-I Lu: None

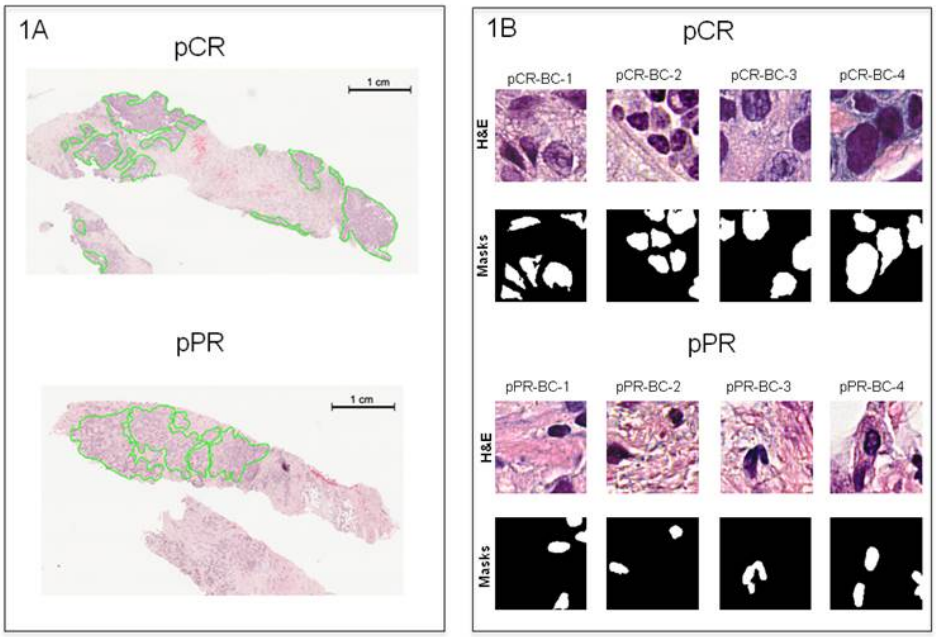
Background: Neoadjuvant chemotherapy (NAC) is used to treat patients with high-risk breast cancer and enables pathological assessment of the tumor response. The tumor response can be classified as either a pathological partial response (pPR) or pathological complete response (pCR), with pCR defined as eradication of invasive tumor in breast and lymph nodes and associated with significantly better survival. Given that only 35% of patients achieve a pCR, predicting the response to NAC remains a significant clinical challenge. The objective of this proof of principle study was to determine if automatic tumor nuclei detection using a digital pathology platform and artificial intelligence (AI) could predict response to NAC.

Design: From a retrospective cohort of breast cancer patients receiving NAC between 2010-2017 at our institution, pre-NAC H&E-stained breast core biopsies were selected from 22 pCR cases and 21 pPR cases. A breast pathologist manually annotated invasive carcinoma on scanned whole slide images to serve as the ground truth to train an AI algorithm (Figure 1A). Convolutional neural networks, with a U-Net architecture, were used to decompose images into tumour components including tumour nuclei (Figure 1B).

Results: Confusion matrix assessment showed 96% accuracy in detection of tumor nuclei, confirming the validity of our AI algorithm. Between the pCR and pPR groups, there were no significant differences in age, menopausal status, tumor size, multifocality, grade, proportion of triple negative tumors, type of chemotherapy or number of cycles received. However, the pCR group had significantly fewer ER/PR+ tumors (46% vs. 76%) and more HER2+ tumors (77% vs. 48%). The average tumor nuclei count assessed by our AI

algorithm was significantly higher in the pCR group (6.08 ± 1.77 vs. 5.10 ± 1.16, $p < 0.05$). Multivariate logistic regression was then performed to control for identified confounders including ER/PR status and HER2 status. Overall the model was significant ($\chi^2=18.446$, $p < 0.001$) and correctly classified 77% of cases. For each additional tumor nucleus detected, the likelihood of achieving pCR was approximately doubled (OR = 1.95, 95% CI: 1.01 – 3.76).

Figure 1 - 150



Conclusions: Automated tumor nuclei detection using digital pathology and AI can successfully predict pCR post-NAC in patients with breast cancer. Future research taking into account additional tumor and micro-environmental features will allow for development of increasingly accurate tools to predict tumor response.

151 LEF1 Expression is Associated with Lymph Node Metastasis in Micropapillary Carcinoma of Breast

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Disclosures: Darin Dolezal: None; Padmini Manrai: None; Lori Charette: None; Xuchen Zhang: None; Malini Harigopal: None

Background: Micropapillary carcinoma (MPC) is a rare, morphologically distinctive breast tumor composed of small clusters that display inverted polarity and loss of stromal adhesion. Patients with MPC present with high rates of lymph node metastasis (LNM) and poor prognosis. While aberrant WNT signaling has been widely implicated in tumor progression, its role in MPC is unclear. We explored WNT activity in MPC by examining the signaling components beta-catenin (CTNNB1) and lymphoid enhancer binding factor 1 (LEF1).

Design: We assessed CTNNB1 and LEF1 nuclear expression by IHC in 50 breast carcinomas using a tissue microarray (TMA) enriched for MPC, and in resection specimens from 15 cases of MPC ranging in pT (pT1, n=7; pT2, n=4; pT3, n=4) and N stage (N0/N1(mi), n=7, N1, n=5, N2/N3, n=3). CTNNB1 and LEF1 staining in MPC and in adjacent invasive ductal carcinoma (IDC) were evaluated by H-score (H=intensity score 0-3 x % cells staining=0-300). For comparison we assessed expression of MYC, EMA, and E-cadherin.

Results: LEF1 nuclear expression was observed in the majority of MPC in the TMA (20/28, 71%, H-score: 87, range 5-200) and in resections (11/15, 73%, H-score: 59, range 5-200) but only rarely in IDC (TMA: 1/22, 4%, H-score: 80, p<.005; IDC[adjacent] in resections: 1/11, 9%, H-score: 10, p<.005). In resections, LEF1 expression was associated with increased tumor size (LEF1+ tumors had average size of 4.8 cm vs. LEF1- tumors avg. 1.35 cm; p<.05), increased lymphovascular invasion (LVI) (10/11 LEF1+ tumors displayed LVI vs. 0/4 LEF- tumors, p<.05), and increased LNM (9/11 LEF1+ tumors displayed LNM vs. 1/4 LEF- tumors, p<.05). LEF1 expression was also observed in MPC-LVI foci from all ten tumors displaying MPC-LVI, as well as in MPC-LNM deposits from 4/5 positive N1 lymph nodes (H avg=137.5). No MPC tumors displayed nuclear accumulation of CTNNB1 in TMA or resections (staining was membranous). The beta-catenin/LEF1 complex promotes transcription of growth regulators such as MYC; we observed no MYC expression in MPC. E-Cadherin levels (at

intercellular membranes and in cytoplasmic vesicles) were reduced (while EMA staining was unchanged) in LEF1+ MPC (n=41/50 tumor clusters) vs. LEF1- MPC (n=5/50, p<.005), suggesting reduced cellular adhesion.

Conclusions: Our study shows increased nuclear expression of LEF1, but not CTNNB1, in MPC. Upregulation of LEF1 correlates with increased tumor size, LVI, and LNM in MPC. We suggest that LEF1 may be a useful ancillary test for predicting LVI and LNM in MPC.

152 Pleomorphic Lobular Carcinoma In Situ's Expression of Targetable Hormone Receptors and Predictive Markers, Evidence for Necessity of Testing

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Disclosures: Reza Eshraghi: None; Tiansheng Shen: None; Indu Agarwal: None; Jorge Novo: None; Luis Blanco: None; Jennifer Pincus: None

Background: Pleomorphic lobular carcinoma in situ (PLCIS) is a variant of lobular carcinoma in situ, with an inadequately characterized immunophenotype, behavior, and management. The expression of hormone receptors ER, PR, and HER2 are powerful prognostic and predictive markers in breast cancer. The expression of tumor suppressor p53 in breast cancer cells and the expression of transcription factor FOXP3 in both tumor cells and tumor infiltrating lymphocytes (TILs) are features associated with aggressive behavior. We investigated the expression of these markers in PLCIS to gain a better understanding of the clinical and biological behaviors of this disease.

Design: The pathology database was searched to identify PLCIS cases diagnosed between 2014 and 2019, along with clinicopathologic characteristics. Expression of ER, PR, p53, and FOXP3 were assessed by IHC. HER2 equivocal (score 2+) cases were evaluated by FISH. ER, PR, and HER2 were graded using ACSO-CAP guidelines. p53 was positive when >10% of tumor cells had nuclear immunoreactivity, and FOXP3 was positive when ≥ 1% of tumor cells or TILs had nuclear immunoreactivity.

Results: Thirty-one cases were identified in female patients ages 38 to 89 (median age = 63). Twenty-seven cases (27/31, 87%) were positive for ER, 20/31 (65%) were positive for PR, 4/30 (13%) had overexpression of HER2 (score 3+), 5/30 were HER2 negative (score 1+ or 0), and 21/30 (70%) were HER2 equivocal (score 2+) by IHC. Of the equivocal cases, 10% were HER2 FISH positive (2/21) and 90% were HER2 FISH negative (90%). p53 was positive in 16/29 cases (55%). FOXP3 was positive in TILs in 12/29 cases (41%), but was negative in all cases of PLCIS (<1%). Invasive lobular carcinoma (ILC) was present in 13/31 cases (42%) and DCIS was present in 3/31 cases (10%). Eight of the PLCIS with ILC cases were FOXP3 TIL positive (8/12, 67%) and all were negative in the tumor cells (<1%). Of these ILCs, two cases were positive for p53, one case was positive for HER2, and the remainder were HER2 negative

Total 31 cases	**ER (%;31)**	**PR(%;31)**	**HER2(30)**	**p53(%,30)**	**Foxp3 (%;29) in PLCIS**	**Foxp3 (%;29) in Tils**	**Dx**	**AGE**
Positive	**27+ (87%; from 15-100%; mean:78)**	**20+ (68%; 1-90%; mean:43)**	**4+(13%)**	**16+ (53%; 10-90%; mean:35%))**	**10+(34%;1-2%)**	**12 with Tils (all +; 1-30%; mean:11%)**		
Equivocal		**5 Low+**	**21 (70%)**					
Negative	**3- (13%)**	**11- (32%)**	**5(17%)**	**14(47%)**	**19-(66%)**			
	95; S	5; W	2+	5; N	<1	no Tils	PLCIS, CLCIS	70
	100; S	5; M	1+	5; N	0	5	PLCIS, CLCIS	59
	60;W-M	1; W-M	3+	<1; N	unreadable	unreadable	PLCIS, CLCIS, ILC	62
	<1; N	0; N	2+ (ILC FISH-)	20; P (diff from ILC)	<1	no Tils	PLCIS, CLCIS, ILC	89
	<1 (<10% ILC)	0 (5 ILC)	2+ (ILC FISH +)	90;P	1-2%	15	PLCIS, ILC	69

	20;W-M	<1;N	3	30;P	2%	5	PLCIS, CLCIS, ILC	81
	95; S	1; W-M	2+	5;N	0	5	PLCIS, CLCIS	59
	0	0	No tissue	No tissue	No tissue	No tissue	PLCIS, CLCIS, DCIS	58
	90;M-S	60;S	2+	95;P	<1	no Tils	PLCIS, CLCIS	64
	95; S	70;M-S	1+	1;N	0	no Tils	PLCIS, ILC	73
	60;S	0	1+	20;P	<1	no Tils	PLCIS, CLCIS	73
	100; S	10;M-S	2+	20; P	0	no Tils	PLCIS, CLCIS	59
	95;S	0	1+	0;N	1	5	PLCIS, ILC	58
	80;S	50;S	2+	30;P	<1	no Tils	PLCIS, CLCIS	40
	95;S	80;M-S	2+ (ILC 1+)	5;N	<1	30	PLCIS, ILC	38
	40;M	0	2+	<1;N	1	no Tils	PLCIS, CLCIS	60
	90;W-M	<1;N (ILC 75)	2+ (ILC 0)	20;P (ILC <1)	1	no Tils	PLCIS, ILC	58
	15;W-M	0	3+	5; N	1	no Tils	PLCIS, CLCIS	70
	95;M-S	90;S (ILC<5)	1+	<1;N	0	no Tils	PLCIS, ILC	48
	100; S	10;M-S	2+	10; P	<1	no Tils	PLCIS, CLCIS, DCIS	59
	90;S	75;M-S	2+	75;P	0	no Tils	PLCIS, LCIS	80
	70;M	60;M	2+	25; P	<1	no Tils	PLCIS (A), CLCIS (B), DCIS(B)	50
	50;M	90;S	2+	15; P	0	no Tils	PLCIS, CLCIS	56
	95;S	30;S	2+ (ILC FISH+)	<1;N	0	20	PLCIS, CLCIS, ILC	74
	95;S	60;W-M	2+	25;P	0	5	PLCIS (focal), CLCIS	73
	60;S	5	2+	40;P	0	no Tils	PLCIS (focal), CLCIS	73
	0	0	2+(ILC FISH-)	5;N	1	1	PLCIS, CLCIS, ILC	64
	30;M-S	0	3+	30;P	1	25	PLCIS	65
	90; M-S	70; M-S	2+(ILC FISH-)	5; N	1	5	PLCIS, CLCIS, ILC	46
	95; S	10; M	2+ (ILC FISH Equv)	<1;N	1	5	PLCIS, CLCIS, ILC	73
	95; S	75%; M	2+	20; P	0	no Tils	PLCIS	65

Conclusions: Our results demonstrate that PLCIS has an unfavorable IHC profile (decreased PR expression, overexpression of HER2, and increased expression of p53). Notably, FOXP3 expression in TILs correlated with more aggressive behavior and the presence of ILC. In PLCIS, treatment targetable markers associated with a poorer prognosis (HER2, p53, and FOXP3) are overexpressed and should be considered as primary markers to assist with treatment decisions

153 Machine Learning Techniques Supplementing Conventional Data Analytics to Identify Cohorts of Women with Screen Detected Breast Cancer Who May Safely Avoid Axillary Surgery

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Disclosures: Gelareh Farshid: None; Robert Whitfield: None; Melissa Bochner: None; Suzanne Edwards: None; Malcolm Pradhan: None

Background: De-escalation strategies are a focus of current breast cancer research. The IBCSG trial questioned the significance of micrometastases and the Z0011 trial suggested women with <3 positive SN having BCS and whole breast irradiation may avoid axillary lymph node dissection. Considering the favourable biology of screen-detected breast cancer, our aim is to determine pre-operative factors that identify cohorts who may safely avoid axillary surgery.

Design: After IRB approval, we searched our database for invasive breast cancers detected by population based mammographic screening. A range of demographic, imaging, histologic and biomarker data were assessed to predict nodal involvement with conventional statistics and machine learning techniques of XGBoost, CART and logistic regression software.

Results: Between Jan 2012-Feb 2017, 374 women, mean age 63.0 years, 96% asymptomatic, are included. Imaging size was ≤15mm in 242 (65.1%). Axillary ultrasound (AUS), performed in 262 (70.1%) was abnormal in 53 (14.4%). Axillary FNA, performed after abnormal AUS, was abnormal in 30 (11.4%) women. Histology grade was G1: 88 (23.5%); G2: 163 (43.6%) & G3: 118 (31.6%). Overall, 272 (73.9%) cancers were N0; 96 (26.1%) had ≥1 positive node.

Univariate log binomial regression analysis revealed significant associations between nodal disease and age, breast symptoms, imaging morphology, location, imaging size (binary, 15mm threshold), AUS, grade, ER, PR, Ki-67, HER2, molecular subtype and histology subtype. Multivariable models retained axillary assessment and imaging size as predictive of nodal involvement. For machine learning models, the data were split randomly into a training set (n=284) and a test set (n=47). The 'boosted tree' model outperformed other machine learning techniques with an overall accuracy of 80.3%. The most significant factors were again identified as imaging size and axillary assessment.

On the basis of this combined analysis, we have identified women with grade 1 cancers, ≤15mm on imaging, with normal AUS or biopsy negative axillary assessment, as having a 4.17% chance of nodal disease. This group comprises 18.3% of women with available axillary assessment data.

Table 1: Predicting Nodal Involvement from Imaging Size, Axillary Ultrasound and Histologic Grade

	Grade 1		Grade 2		Grade 3	
Axillary Ultrasound	**≤15mm**	**>15mm**	**≤15mm**	**>15mm**	**≤15mm**	**>15mm**
Normal, no bx	2/43 (4.4%)	2/7 (28.6%)	11/61 (18.0%)	15/31 (53.5%)	6/38 (15.8%)	14/26 (53.9%)
Abnormal, bx normal	0/5 (0%)	0/2 (0%)	1/4 (25.0%)	0/3 (0%)	2/3 (66.7%)	0/2 (0%)
Abnormal, bx abnormal	0/3 (0%)	NA	1/3 (33.3%)	6/6 (100%)	2/3 (66.7%)	13/14 (92.9%)
Abnormal, no bx	NA	NA	NA	1/1 (100%)	NA	1/1 (100%)
Not assessed	0/18 (0%)	0/5 (0%)	5/32 (15.6%)	6/17 (29.3%)	2/14 (14.3%)	6/12 (50%)
Overall	2/71 (2.8%)	2/14 (14.3%)	18/100 (18.0%)	28/58 (48.3%)	12/58 (20.7%)	34/55 (61.8%)

Figure 1 - 153

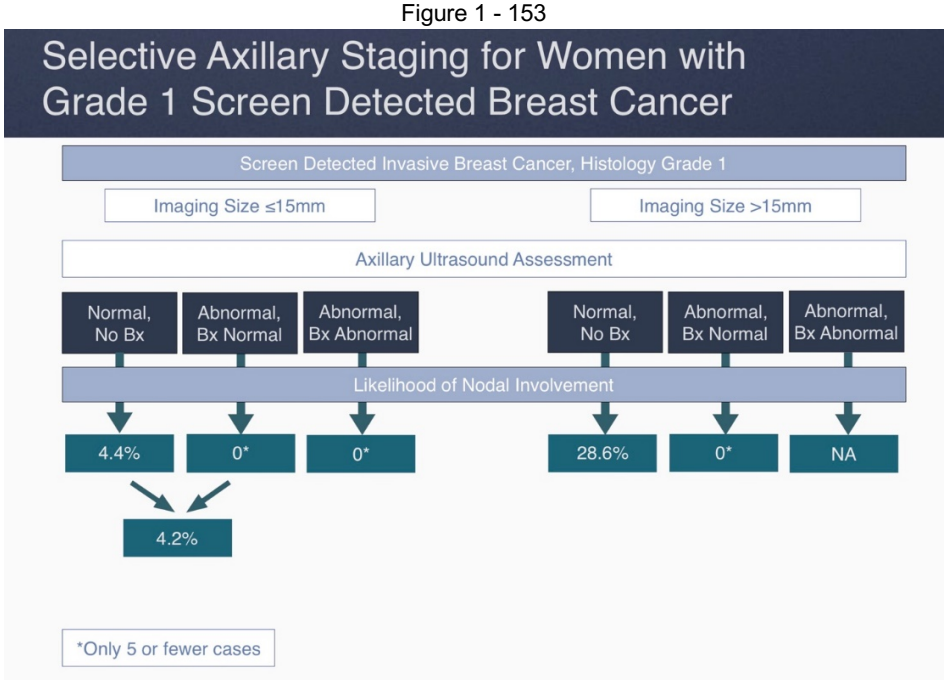
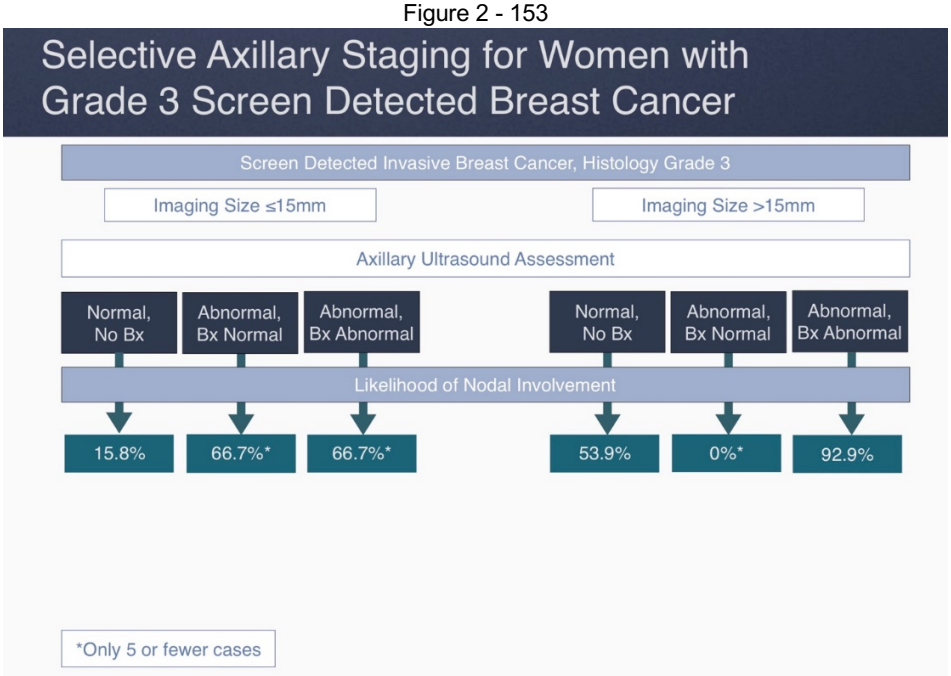


Figure 2 - 153



Conclusions: The chances of nodal involvement in women with grade 1 screen-detected breast cancers, ≤15mm on imaging and negative axillary ultrasound/FNA assessment is less than the commonly accepted 5% false negative rate of sentinel node biopsy. Avoidance of routine axillary surgery may be plausible for these women.

154 Androgen Receptor Splicing Variant-7 in Breast Carcinoma: Clinical and Pathologic Correlations

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Disclosures: Donna Ferguson: None; Douglas Mata: None; Sarat Chandarlapaty: *Consultant*, BMS; *Consultant*, Novartis; *Consultant*, Eli Lilly; *Consultant*, Sermonix; *Consultant*, Revolutions Medicine; Edi Brogi: None; Marc Ladanyi: None; Maria Arcila: *Speaker*, Biocartis; *Speaker*, invivoscribe; Ryma Benayed: None; Dara Ross: None

Background: The role of androgen receptor (AR) inhibitor therapy in AR-positive breast cancer (BC) is evolving. AR splicing variant-7 (AR-V7) is a truncated variant of AR, previously described in prostate cancer, which leads to constitutive activation of AR signaling and resistance to AR inhibitor therapy. AR-V7 has also been identified in hormone-receptor positive and negative BCs (primary and metastatic). Use of aromatase inhibitors (AI) in ER-positive BC, which halts the production of estrogen from androgen, may upregulate AR signaling and lead to increased AR-V7. We assess the clinicopathologic features of AR-V7 in BC to evaluate if screening certain BC prior to trial enrollment is warranted.

Design: BC with AR and ER immunohistochemistry and clinical DNA and/or RNA sequencing results were screened for fusions. After DNA sequencing, BC reflexed to RNA sequencing during the period studied (9/2018-6/2019) included triple-negative tumors with no driver and ER-positive/ESR1 wildtype tumors progressing on therapy. All BC with AR-V7 identified by RNA sequencing were included, as well as a subset of AR-V7 negative specimens with various receptor profiles.

Results: Among 52 FFPE cases (52 with RNA sequencing, 51 with DNA sequencing and 51 with IHC), 35% (18/52) were AR-V7 positive and 65% (34/52) AR-V7 negative. AR-V7 did not alter AR IHC expression, as all AR-V7 positive cases were AR IHC positive. Table 1 lists cohort histology and receptor profiles. AR-V7-positive BC were more likely to have mutations in *AKT1* ($p = 0.03$) and *FANCA* ($p = 0.03$). Of 18 AR-V7 positive BC, 50% (3/6) of primary and 42% (5/12) of metastatic/recurrent tumors had prior exposure to AI for treatment of same or a prior hormone-positive BC. Apocrine morphology was observed in 39% (7/18) of AR-V7 positive BC and 3% (1/34) of AR-V7 negative BC ($p = 0.002$). In all, only 28% (5/12 metastatic/recurrent; 0/6 primary) AR-V7 tumors were both negative for apocrine morphology and AI naïve at the time of AR-V7 detection (Figure 1).

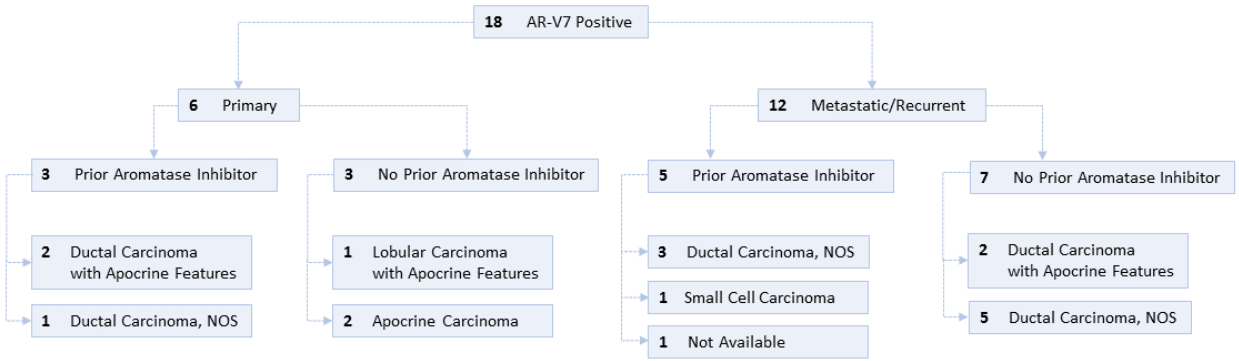
	AR-V7 positive (n=18)	**AR-V7 negative (n=34)**
Age at BC Diagnosis, Median (Range), Years (n = 52)	53.3 (31.9 – 72.9)	56.8 (31.3 – 82.7)
Sample Type (n = 52)		
Primary, Post ET[1], including AI	50.0% (3/6)	0.0% (0/12)
Primary, Post ET, SERM only	0.0% (0/6)	8.3% (1/12)
Primary, No ET	50.0% (3/6)	91.7% (11/12)
Metastatic/Recurrent, Post ET, including AI	41.7% (5/12)	59.0% (13/22)
Metastatic/Recurrent, Post ET, SERM only	8.3% (1/12)	0.0% (0/12)
Metastatic/Recurrent, No ET	50.0% (6/12)	40.9% (9/22)
Histologic Subtype (n = 47)[2]		
Ductal Carcinoma, NOS	56.3% (9/16)	80.0% (24/30)
Apocrine Carcinoma	12.5% (2/16)	0.0% 0/30
Ductal Carcinoma with Apocrine Features	25.0% (4/16)	3.3% (1/30)
Lobular Carcinoma with Apocrine Features	6.3% (1/16)	0.0% (0/30)
Other	6.3% (1/16)	16.7% (5/30)
Grade (n = 47)[2]		
Well to Moderately Differentiated	0.0% (0/16)	3.3% (1/30)
Moderately Differentiated	25.0% (4/16)	23.3% (7/30)
Moderately to Poorly Differentiated	18.8% (3/16)	0.0% (0/30)
Poorly Differentiated	56.3% (9/16)	73.3% (22/30)
Receptor Status (n = 52)		
ER positive, AR positive	33.3% (6/18)	29.4% (10/34)
ER negative, AR positive	66.6% (12/18)	26.5% (9/34)
ER negative, AR negative[3]	0.0% (0/18)	44.1% (15/34)

[1]ET: Endocrine therapy, including selective estrogen receptor modulators (SERM), selective estrogen receptor degraders (SERD), and aromatase inhibitors (AI)

[2]Subtype and grade based on primary tumor, information available for n=47

[3]1 case HER2-positive

Figure 1 - 154



Conclusions: AR-V7 is present in a high proportion of BC with apocrine morphology and from patients with prior AI therapy. Screening for AR-V7 in patients with AR IHC positive BC, apocrine morphology and/or prior AI therapy may be prudent prior to treatment with AR inhibitors.

155 Routine Excision is Not Necessary for Mucocele-Like Lesions of the Breast

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Disclosures: Alexander Filatenkov: None; Sunati Sahoo: None; Venetia Sarode: None; Yan Peng: None; Yisheng Fang: None; Helena Hwang: None

Background: Mucocele-like lesions (MLL) of the breast are characterized by extravasated acellular mucin pools adjacent to mucin filled dilated ducts. They usually present as mammographic calcifications. The incidence of MLL in core needle biopsies (CNB) is low and optimal management is not standardized, with the literature showing varying rates of upgrade on excision. The purpose of this study is to help determine optimal management of patients diagnosed with MLL on core biopsy.

Design: After institutional review board approval, the databases of two different hospitals were searched for MLL for the period of 2008-2018. All core biopsies showing MLL were identified; any MLL with ADH or carcinoma (ductal carcinoma in situ (DCIS) or invasive carcinoma) were excluded. Data that was collected included the reason for CNB (calcifications versus mass), the method of biopsy (ultrasound, stereotactic, MRI), needle gauge, and number of cores taken. Patient data, including demographics, subsequent excisions, length of follow up, and whether the patient developed subsequent breast cancer was also recorded.

Results: 46 CNB with MLL were identified. 38 cases were biopsied for calcifications and 8 were biopsied for a mass, with or without calcifications. Of the 46 cases, 21 underwent excision with no upgrades to DCIS or invasive carcinoma. 22 of the 24 patients (one patient had two CNBs) who did not undergo excision had follow up ranging from 9-95 months (median=42 months) and none developed breast carcinoma (neither DCIS nor invasive carcinoma).

Conclusions: This study shows no upgrade of MLL diagnosed on CNB to breast carcinoma (DCIS and invasive carcinoma). Of the patients with follow up who did not undergo excision of MLL, none developed breast carcinoma. While the number of cases in this study is small, this study argues against routine excision of MLL found on CNB.

156 Androgen Receptor Expression and Tumor Immune Microenvironment in Ductal Carcinoma in Situ (DCIS) of the Breast

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Disclosures: Brian Finkelman: None; Julianne Ubago: None; Luis Blanco: None; K. P. Siziopikou: None

Background: The androgen receptor (AR) pathway has emerged as a potential therapeutic target of interest in breast cancer. Overall, 60-80% of breast carcinomas are AR+, with heterogeneous expression in different molecular subtypes. Recent studies also suggest that AR overexpression may be inversely correlated with both tumor infiltrating lymphocyte (TIL) levels and PD-L1 expression in TILs in HER2+ breast carcinoma. AR expression in ductal carcinoma in situ (DCIS), however, is poorly understood. Furthermore, to our knowledge, no data are available on how AR expression in DCIS may interact with the tumor immune microenvironment.

Design: Our population consisted of patients with pure DCIS, treated with definitive surgical therapy at our institution (2008-12). DCIS Molecular subtypes were defined as: Luminal A (ER+/HER2-), Luminal B (ER+/HER2+), HER2-enriched (ER-/HER2+), and basal-like (ER-/HER2-). Tissue microarrays were constructed with 3 cores/case to account for tumor heterogeneity. HER2 equivocal (score 2+) cases or with insufficient material to test AR were excluded. AR positivity was defined as ≥10% staining in tumor cells. TIL levels were graded semiquantitatively as absent, mild (<10%), moderate (10-50%) and severe (>50%). PD-L1 was considered positive if ≥1% of TILs showed staining. Associations between variables of interest were assessed via Fisher's exact test.

Results: 96 patients with pure DCIS were identified (mean age 56 years, range 39-85). Nuclear grade 3 was seen in 57%, grade 2 in 34%, and grade 1 in 8% of lesions. The large majority of lesions were AR+ (89/96, 93%). AR positivity was seen in 48/51 (94%) Luminal A lesions, 18/19 (95%) Luminal B lesions, 14/15 (93%) HER2-enriched lesions, and 4/6 (67%) basal-like lesions. Of interest, 23/96 lesions (24%) were ER-/AR+. No significant difference between AR+ vs AR- lesions was seen in either the level of TILs (40/83, 48%, vs 5/6, 83%, P = 0.11) or PD-L1 positivity by TILs (54% vs 50% P > 0.99).

Conclusions: 1) AR was expressed in the majority of DCIS cases, comparable to that seen in invasive carcinoma. 2) Nearly one fourth of patients were AR+/ER-; this group could potentially be expected to benefit from anti-androgen therapy. 3) While we did not observe a significant effect of AR expression on either TIL levels or PD-L1 expression, more data are needed to definitively assess the impact of AR expression on the immune microenvironment in DCIS, particularly in HER2+ cases.

157 Utility of p53 Immunostaining in Predicting Multigene Signatures Risk Category Results and Molecular Subtypes in a Series of Estrogen Receptor Positive and HER2 Negative Early Breast Cancers

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Disclosures: Giuseppe Floris: None; Laurence Slembrouck: None; Mieke Van Bockstal: None; Laura Gadeyne: None; Anne-Sophie Van Rompuy: None; Cecile Colpaert: None; Sanne Verbeek: None; Claire Bourgain: None; Kevin Punie: None; Hans Wildiers: None; Christine Desmedt: None; Ann Smeets: None; Ines Nevelsteen: None; Sara Vander Borght: None; Isabelle Vanden Bempt: None; Patrick Neven: None

Background: Luminal-B breast cancers are characterized by worse prognosis and, more frequently carry *TP53* mutations as compared to Luminal-A tumors. Aberrant p53 (AB^{p53}) protein expression like a diffuse and strong nuclear expression, is predictive for pathogenic *TP53* mutations. We studied the utility of p53 immunostaining in predicting the results of 3 commercially available multi gene signatures (MGS) frequently used to assess the risk of recurrence of Luminal early breast cancers.

Design: This retrospective study included 179 primary operable $ER^{Pos}/HER2^{Neg}$ breast cancers diagnosed at University Hospitals Leuven between June 2013 and May 2019, that were analyzed either by MammaPrint®&BluePrint®(MP/BP; n=78), OncotypeDX® (ODX; n=44) or Prosigna/PAM50® (PAM50; n=57) MGS. Negative MP-index, RS score ≥26 and recurrence risk ≥11% were considered as high risk (HiR) categories in MP, ODX and PAM50, respectively. Intrinsic molecular breast cancer subtypes were provided by the combination MP/BP and the PAM50 assay. Whole-slide sections of the same paraffine block used for MGS testing were stained with anti-human p53 antibody (DAKO; clone DO-7). Patterns of AB^{p53} expression are shown in Figure 1. Statistics was by Fisher's exact test

Results: Twenty samples were excluded for insufficient residual tissue or poor-quality internal control (MP=9, ODX=4, PAM50=5). The remaining 159 showed an AB^{p53} pattern in 13% (n= 20) of the cases; with aberrant nuclear pattern in the majority (n=16). MGS HiR was observed in 34% (n=23/67), 28% (n=11/40) and 61% (n=32/52) of patients tested with MP, ODX and PAM50 respectively. Only MP HiR

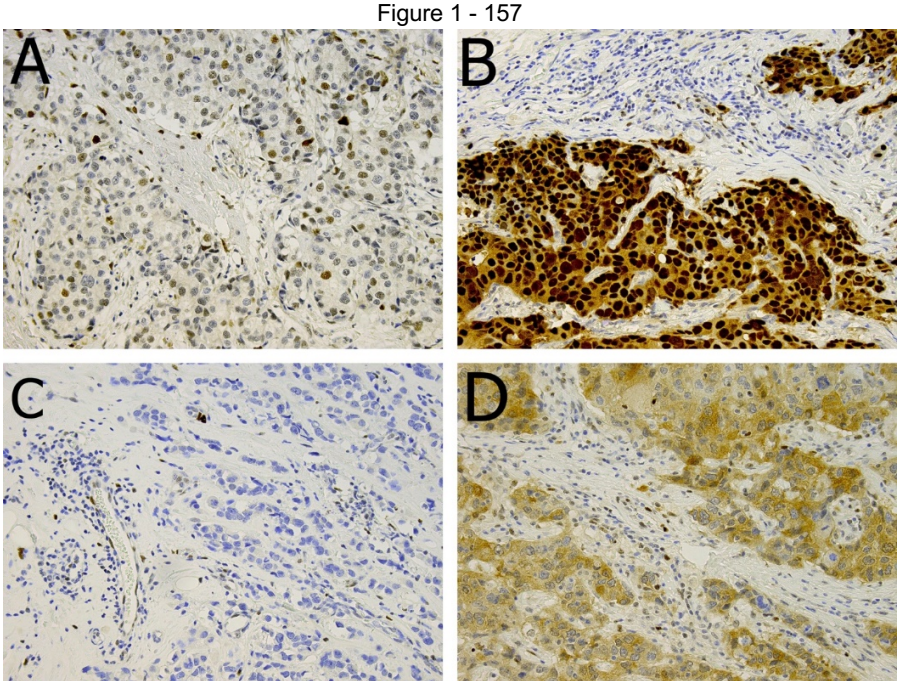
showed a significant positive association with AB^{p53} (p<0,01). The BP/MP assay classified 44 (66%), 22 (33%) and 1 (1%) tumors respectively as luminal-A, B and Basal subtype. The PAM50 classified 32 (61%), 17 (33%), 1 (2%) and 2 (4%) tumors respectively as luminal-A, B, HER2 and Basal subtype. We found significant positive association between AB^{p53} and BP/MP^{LumB} (p<0,01). No significant associations were found between AB^{p53} and ODX or PAM50 (Table 1)

MGS			P53 patterns by IHC		Total
			Wild type	Aberrant	(n=159)
Mammaprint (n=67)	Low-Risk	Number of cases (Mol.subtype)	42 *(LA=42)*	2 *(LA=2)*	44 *(LA=44)*
		% within MGS	93.2%	6.8%	100%
	High-Risk	Number of cases (Mol.subtype)	16 *(LB=16)*	7 *(LB=6; BA=1)*	23 *(LB=22; BA=1)*
		% within MGS	69.6%	30.4%	100%
Prosigna (n=52)	<11%RR	Number of cases (Mol.subtype)	19 *(LA=17; LB=2)*	1 *(LA=1)*	20 *(LA=18; LB=2)*
		% within MGS	95%	5%	100%
	≥11%RR	Number of cases (Mol.subtype)	30 *(LA=14; LB=14; H2=1; BA=1)*	2 *(LB=1; BA=1)*	32 *(LA=14; LB=15; H2=1; BA=2)*
		% within MGS	93.7%	6.3%	100%
Oncotype Dx (n=40)	<26RS	Number of cases	24	5	29
		% within MGS	82.8%	17.2%	100%
	≥26RS	Number of cases	8	3	11
		% within MGS	72.7%	27.3%	100%

Table1: *Patterns of p53 expression in relation to risk categories and intrinsic molecular subtypes as assessed by commercially available multi gene signatures.* The association between aberrant p53 and high-risk category was significant only in MP tested tumors (p<0,03 and for Prosigna and OncotypeDx respectively p=1 and p=0,660). In Prosigna tested tumors luminal-B subtype and non-luminal tumors were more frequently associated with high-risk category independent of p53 status. Aberrant p53 status was significantly associated with luminal-B subtype only in MammaPrint/BluePrint tested tumors. Due to the small number of cases the association between aberrant p53 and non-luminal tumors was not assessed. Abbreviations: MGS= multi gene signature; IHC= immunohistochemistry; mol. = molecular; RR= recurrence risk; RS= recurrence score; LA= luminal A; LB= luminal B; H2= HER2 enriched; BA= basal. No Normal-like tumors were found with Prosigna.

Figure 1: *Patterns of p53 expression by immunohistochemistry. A) wild type pattern was characterized by a variable p53 nuclear expression at various intensities; B) the aberrant-positive pattern was characterized by strong and intense nuclear staining in >95% of the tumor cells; C) the aberrant null pattern was characterized by complete lack of nuclear p53 expression in >95% of the tumor cells against a background of sparse positive stromal cells; D) aberrant cytoplasmic pattern was defined as cytoplasm expression in >95% of the tumor cells without clear nuclear expression.*

Figure 1 - 157



Conclusions: Aberrant p53 staining patterns are significantly associated with MP high risk category and luminal-B BP/MP subtyping. Integration of p53 immunostaining in multiple pathologic parameters aiming at predicting MGS results warrants further study.

158 A Single Institution Experience with Breast Cancer Patients with CDH1 Mutations

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Background: Inherited mutations in the *CDH1* gene increase the risk of developing hereditary diffuse gastric cancer (HDGC) and lobular breast cancer (Lob Br CA).The lifetime risk of developing Lob Br CA in patients (pts) with *CDH1* mutation is estimated at 39-52% but given the rarity of these mutations, Br lesions from this population have not been well characterized. In this study, we describe the clinicopathologic features of pts with *CDH1* mutations.

Design: All pts with deleterious *CDH1* mutations and Br pathology, identified from an institutional registry, were included. Clinical and pathologic features were evaluated.

Results: Nine pts with Br pathology were identified from a total of 41 pts known to have a deleterious *CDH1* mutation. All pts were female, ages 34 to 61. None were of Ashkenazi descent, 6 (67%) were Caucasian, 2 (22%) were Asian, and 1 (11%) was of African descent. Eight (89%) pts came to medical attention due to Br masses and were subsequently diagnosed with Br CA that prompted multi-gene panel testing. The 9th pt underwent panel testing due to a strong family history (FH) of Br CA. Seven distinct *CDH1* mutations are represented in these 9 pts (Table). None of the pts presented with gastrointestinal issues or prior gastric cancer (GC). Two (22%) had a FH of GC, while 7 (78%) had a FH of Br CA. Five (56%) have subsequently undergone gastrectomy; 3 (33%) had pT1a GC of signet ring cell type and 2 (22%) had negative gastrectomies. The remaining 4 (44%) have had only negative gastric biopsies. Eight (89%) had invasive Br CA diagnosed with morphologies including classic Lob (n=4), pleomorphic Lob (n=2), and ductal (n=2). One pt had only atypical Lob hyperplasia on Br biopsy. pT ranged from pT1b-pT2 with 4 pts being pN1a. All cancers were ER and PgR positive, with only one being HER2 positive. IHC for E-cadherin was negative in the classic Lob and pleomorphic Lob, while the ductal morphology was positive.

Patient	Breast Pathology	*CDH1* Mutation
1	Invasive lobular carcinoma, classical	c.1137 G>A
2	Invasive lobular carcinoma, classical	c.1008 G>A
3	Invasive lobular carcinoma, pleomorphic	c.715 G>A
4	Invasive ductal carcinoma	c.208 dupT
5	Atypical lobular hyperplasia	c.640 del
6	Invasive lobular carcinoma, pleomorphic	c.1008 G>A
7	Invasive lobular carcinoma, classical	c.532 G>C
8	Invasive lobular carcinoma, classical	c.1137 G>A
9	Invasive ductal carcinoma	5'UTR_EX2del

Conclusions: Although small, our study raises the possibility that some *CDH1* mutations lead to Br CA as the initial and dominant clinical presentation. This is also reflected in the pts' FHs, where Br CA was more common than GC. While the majority of Br CA arising in pts with *CDH1* mutations are of Lob phenotype, E-cadherin intact invasive ductal carcinoma was also seen, pointing out that Br lesions may also be true true and unrelated in this pt population. Moreover, these pts' germline mutations were identified on multi-gene panel testing, supporting the broadening use of these assays.

159 The Importance of Histologic Subtypes of Triple Negative Breast Cancers on Immune Cell Density and PD-L1 Expression

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Disclosures: Rochelle Freire: None; Carmen Gomez-Fernandez: None; Sara Salehiazar: None; Merce Jorda: None; Nicolas Millan: None; Yamac Akgun: None; M Nadji: None

Background: Immunotherapy with checkpoint inhibitors using PD-L1 targets on tumor cells (TC) and infiltrating immune cells (IC) offers potential new treatment strategies for triple negative breast cancers (TNBC). However, TNBC are a heterogeneous group of tumors with diverse histologic subtypes and varying IC in the tumor microenvironment. Identifying the subset of TNBC that will most benefit from immune modulation will be required for appropriate patient selection. TNBC include infiltrating ductal carcinoma, not otherwise specified (IDC-NOS), and special subtypes such as metaplastic carcinoma (MET), medullary carcinoma (MED), apocrine carcinoma (APC), and “salivary-like” carcinoma, among others. In this study, we sought to evaluate the expression of PD-L1 in the TC and IC of the special TNBC histologic subtypes.

Design: From 2017 to 2019, there were 103 patients with TNBC in our database, of which special histologic subtypes included 15 MET, 9 MED, and 7 APC. Two pathologists evaluated one representative H&E stained slide for IC density and its corresponding PD-L1 (22C3) stained slide for PD-L1 expression in the TC and IC. Partial or complete circumferential membrane staining for PD-L1 of any intensity was scored as positive in the TC. Fine punctate membrane or diffuse granular staining for PD-L1 was scored as positive in the IC. IC density and PD-L1 staining of IC and TC were scored as follows: <1% (negative), 1-50% (low positive, LP), >50% (high positive, HP).

Results: As expected, the MED had the highest IC density with all cases (9/9) scored as HP. All MET and APC were scored as LP for IC density. For PD-L1 expression, all of the MED demonstrated positive staining of TC and IC, with PD-L1 expression localized primarily in the interface of the TC with the IC. The majority of the MET demonstrated PD-L1 staining of the TC (10/15) and IC (11/15), with most cases scored as LP. None of the APC (0/7) expressed PD-L1 in the TC or IC.

Conclusions: Immune cell density varies considerably among the special histologic subtypes of TNBC. PD-L1 expression in the tumor cells and the immune cells also varies among the special histologic subtypes of TNBC. The apocrine carcinoma subtype of TNBC does not express PD-L1 in the tumor cells or the immune cells. This study underscores the importance of determining the histologic subtype of TNBC before the possibility of immunotherapy is entertained.

160 ESR1 Genetic Alterations and their Association with Clinicopathologic Characteristics in Breast Carcinoma: A Single Academic Institution Experience

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Background: Approximately 70% of breast carcinomas (BC) express estrogen receptor (ER). This is a critical attribute analyzed to determine usage of endocrine therapies for treatment of BCs. ER is encoded by *ESR1*, and genetic alterations, especially activating

mutations, in this gene are associated with resistance to endocrine therapies. Herein, we analyzed an institutional cohort of BCs harboring *ESR1* genetic alterations (mutations and amplifications) and associated clinicopathologic characteristics.

Design: The study included 223 BCs diagnosed between 2013 and 2018 that had undergone FoundationOne CDx testing. Clinicopathologic characteristics were collected and associations were analyzed using Fisher's exact test.

Results: Of the 223 cases of BC (136 ER+ and 87 ER-) analyzed, 13.9% (n=31) had *ESR1* genetic alterations, including 26 mutations and 5 amplifications. All 26 *ESR1*-mutated BCs were ER-positive and its prevalence in ER-positive BCs was 19.1% (26/136). The prevalence of *ESR1* mutations was significantly higher in metastatic ER+ BCs (23%, 23/100) than in primary ER+ BCs (8.3%, 3/36) (p=0.0275). Four cases were HER2 positive with 3 cases in the *ESR1*-amplified group and 1 in the *ESR1*-mutated group (60.0% vs 3.8%, p=0.0006).

All mutations occurred in *ESR1* ligand-binding domain, including Y537S (42.3%; n=11), D538G (38.5%; n=10), L536H (n=2), L536Q (n=2), Y537C (n=2), V534E (n=1) and Y537N (n=1). Three cases had double mutations (D538G/L536Q, D538G/Y537N, and D538G/Y537S).

The most common gene mutations other than *ESR1* in these 31 BCs included *PIK3CA* (35.5%), *FGF3/4/19* (35.5%), *FGFR1/3* (29%), *ZNF703* (29%) and *TP53* (25.8%), with no significant difference from BCs without *ESR1* genetic alterations. However, p53 mutations occurred more frequently in *ESR1*-amplified BCs than in *ESR1*-mutated BCs (80% vs 15.4%, p=0.0025). All patients in this cohort received hormonal therapy (aromatase inhibitors and/or tamoxifen) except one *ESR1*-amplified patient (ER-negative).

Table 1. Clinicopathologic characteristics of 31 breast carcinomas with *ESR1* alterations

		Total *ESR1* genetic alterations (n=31)		**_ESR1_ amplification (n=5)**		***ESR1* mutations (n=26)**		**p value**
Age		53	36-73	59.4	50-70	51.9	36-73	NS
Histologic	IDC	27	87.1%	4	80%	23	88.5%	NS
	ILC	4	12.9%	1	20%	3	11.5%	
Biomarkers	ER+	30	96.8%	4	80.0%	26	100.0%	NS
	ER%	84%	0-100%	77%	0-99%	90%	50-100%	
	PR+	23	74.2%	2	40.0%	21	80.8%	NS
	PR%	41%	0-100%	14%	0-50%	50%	0-100%	
	HER2+	4	12.9%	3	60.0%	1	3.8%	0.0006
Locations	Primary	4	12.9%	1	20.0%	3	11.5%	NS
	Metastatic	27	87.1%	4	80.0%	23	88.5%	
ESR1 mutation type	V534E	1	3.2%	0	0.0%	1	3.8%	NA
	L536H	2	6.5%	0	0.0%	2	7.7%	
	L536Q	2	6.5%	0	0.0%	2	7.7%	
	Y537C	2	6.5%	0	0.0%	2	7.7%	
	Y537N	1	3.2%	0	0.0%	1	3.8%	
	Y537S	11	35.5%	0	0.0%	11	42.3%	
	D538G*	10	32.3%	0	0.0%	10	38.5%	
Other mutations	PIK3CA	11	35.5%	1	20.0%	10	38.5%	NS
	FGF3/4/19	11	35.5%	1	20.0%	10	38.5%	NS
	FGFR1/3	9	29.0%	3	60.0%	6	23.1%	NS
	ZNF703	9	29.0%	3	60.0%	6	23.1%	NS
	P53	8	25.8%	4	80.0%	4	15.4%	0.0025
Hormonal therapy		30	96.8%	4	80.0%	26	100%	NS

Abbreviations: ER, estrogen receptor; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; NA, not applicable; NS, not significant; PR, progesterone receptor.

*Three cases with double mutations: D538G/Y537N, D538G/L536Q, D538G/Y537S.

Conclusions: In this study, 19.1% of ER+ BCs showed *ESR1* activating mutations, suggesting the necessity of developing specific drugs targeting these mutants. Our data also demonstrate *ESR1* mutations occur more frequently in metastatic ER+ BCs than in primary ER+ BCs, and that there are clinicopathologic differences between *ESR1*-mutated and *ESR1*-amplified BCs.

161 Skin Involvement and Ulceration in Otherwise pT1, pT2 or pT3 Breast Carcinomas: Retrospective Review from a Single Institution

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Background: Histomorphologic and clinical evidence of skin involvement by breast carcinoma (BC) dramatically upstages patients to pT4b (skin ulceration and/or ipsilateral macroscopic satellite nodules) or cT4d (clinical findings of erythema and edema involving at least one-third or more of the skin of the breast, inflammatory breast carcinoma). However, few studies extrapolate the significance of skin involvement and ulceration (SU) outside of the setting of inflammatory breast carcinoma (IBC). Khoury et al. suggest that SU may not be an adverse factor (Khoury, 2018); and, Güth et al. suggest pT4a-c categories “should be eliminated” altogether, and to classify tumors based on size (Güth, 2007). In the current study, we investigate the significance of skin involvement in BC based on tumor size and compare it with IBC.

Design: Our institutional pathology database was searched for diagnoses of (1) “breast carcinoma” with (2) “skin” “ulceration”, “extension”, and “erosion”, and (3) the designation "pT4”. The cohort includes cT4d inflammatory breast carcinomas (IBC) and other pT4b BC that did not meet the criteria for IBC. A total of 59 cases met the search criteria (both biopsy and excision specimens), constituting 48 patients from 2001 to 2019. All cases that were pT4b were reclassified by tumor size into pT1c, pT2 or pT3.

Results: Of the 48 patients, original pT classifications included 29 pT4b, 13 IBC, and 6 incompletely staged cases. Reclassifying the pT4b tumors into T1c (n=5), T2 (n=14), and T3 (n=11), did not yield any statistically significant associations ($p < 0.05$) with respect to (1) parameters such as lymphovascular invasion (LVI) or lymph node (LN) positivity, or (2) difference in overall survival between pT1c, pT2 and pT3 with SU, originally denoted as pT4b. However, when comparing IBC versus pT4b, statistically significant associations were noted in the frequency of LVI (p=0.0195) and LN positivity with IBC (p=0.0358), Table 1. Length of follow up ranged from 4 to 133 months.

Table 1: Comparison of Inflammatory and Non-Inflammatory T4 Breast Carcinomas

n=48	Inflammatory	Non-inflammatory
Number of cases	13	35
Size range (mm)	15-140	11-160
Age range	49-94	33-99
Sex (F=Female; M=Male)	11F; 2M	34F; 1M
Laterality (L=Left; R=Right)	5L; 8R	14L; 21R
Invasive, ductal type	11 (85%)	23 (68%)
Invasive, lobular type	0	6 (18%)
Invasive, other types	2 (15%)	5 (15%)
Nottingham grade, I	0	2 (6%)
Nottingham grade, II	5 (38%)	12 (39%)
Nottingham grade, III	8 (62%)	18 (58%)
Lymphovascular invasion (LVI)	13 (100%)	21 (66%)
Ipsilateral macroscopic satellite nodules	5 (38%)	12 (40%)
Positive lymph nodes	12 (100%)	17 (68%)
Skin ulceration (SU)	2 (17%)	9 (31%)

Note: Percentages do not include cases where the data point is not-available/non-applicable.

Conclusions: This series shows that patients with AJCC-defined pT4b BC did not have an association with LVI or LN positivity while IBC did have with both. Although our follow-up is limited by short duration for a subset of patients, if the observed trend for pT4b tumors to behave less aggressively is confirmed, consideration for a change in staging assignment may be warranted.

162 Inhibitor of Differentiation Genes in Breast Cancer: An In Vitro and Clinicopathological Study

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Background: Inhibitor of Differentiation (ID) proteins are a family of four (ID1-4) bHLH transcription factors that lack the DNA binding domain and act by negatively regulating transcription. Their action is essential for embryonic development, whereas later in the adulthood their expression is mostly restricted to a few populations of stem cells. In the last decades, many authors have described their role in the pathogenesis of different neoplasias. However, in breast cancer (BC) the pathogenic role and the clinical significance of ID genes are not fully elucidated.

Design: We included 307 non-consecutive breast carcinomas (19.9% Luminal A, 19.2% Luminal B/HER2-, 15.6% Luminal B/HER2+, 13.4% HER2-enriched and 31.9% TNBL), and 6 BC cell lines representative of all intrinsic subtypes (T47-D, MCF-7, BT-474, SKBR3, MDA-MB231 and MDA-MB-468). The mRNA expression of ID1-4 was analyzed by qRT-PCR using TaqMan® primers and probes. PUM1 and β-actin were used as reference genes and a pool of healthy breast tissue or the non-tumor cell line 184A1 served as control samples. Relative changes in expression were calculated by the $2^{-\Delta\Delta CT}$ and the results were correlated with clinicopathological factors (age, tumor size and grade, vascular invasion, necrosis, immunophenotype, and lymph-node status) and patients' outcome. Significant associations between variables were calculated with χ^2 and Student-T test and differences in Kaplan-Meier survival plots were compared using the log-rank test.

Results: Overexpression of at least one ID gene was found in 50.5% of the samples. ID1 and ID4 were overexpressed mostly in TNBL and HER2-enriched subtypes, whereas overexpression of ID2 and ID3 was more frequently found in luminal tumors. High ID1 and ID4 expression was associated with larger tumor size, histological grade 3, presence of necrosis and vascular invasion, and poorer patients' outcome. *In vitro* studies showed high expression of the four ID genes in all cell lines, including the normal breast 184A1. However, the expression of ID4 was markedly higher in cancer cell lines than in 184A1.

Conclusions: These data support that ID1 and ID4 may act as biomarkers of tumor aggressiveness and worse prognosis in patients with breast cancer. Therefore, they seem potential targets for the development of novel drugs.

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163 Investigating the Use of Convolutional Neural Networks in Diagnosis of Microinvasive Carcinoma of the Breast: Lessons Learned in a Small Dataset

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Disclosures: Matthew Gayhart: None; Matthew Gayhart: None; Lorraine Colon Cartagena: None; Patricija Zot: None; Valentina Robila: None

Background: At our institution, most breast biopsy results are reported within a 24 hour turn-around time (TAT). However, in cases of ductal carcinoma in situ (DCIS) with suspected microinvasion (MI), examination of additional tissue levels and confirmatory stains for myoepithelial markers most often lead to extension of TAT. To this end, we proposed to investigate whether a well-trained deep learning based image classifier may aid as a diagnostic tool to distinguish DCIS from DCIS with MI on routinely stained H&E images.

Design: 92 archival cases of DCIS and 17 cases diagnosed as DCIS with MI were identified. Whole slide imaging was performed on H&E stained slides using a NanoZoomer 2.0HT, at 40x magnification. A total of 561 images (458 DCIS, 104 DCIS with MI) measuring 154x154 pixels were created and randomly divided into a 449 training image subset and a 112 testing image subset. The images were then used to create an image classification model using the ResNet-50 convolutional neural network architecture that would identify MI versus DCIS.

Results: After model training, 112 images (91 DCIS, 21 DCIS with MI) were tested for correct classification. All 91 (100%) images of DCIS only were correctly identified, importantly with no false positives for an invasive component. MI was detected in 14 of 21 (66.7%) images, with an overall accuracy of 93.8%. The lack of MI detection in 7 images may be in part due to more limited training given the low image sample size of DCIS with MI compared to the sample training size of DCIS (104 vs 458). In addition, identifying MI is very challenging on routinely stained H&E slides, even with the help of myoepithelial markers. Therefore, the low image sample size combined with the very challenging diagnosis could explain the low detection rate of MI by the algorithm.

Conclusions: Our results show there is potential for the use of a deep learning based decision support tool to assist in rapidly identifying DCIS with MI on routine H&E stained slides. In our cohort, detection of DCIS is highly accurate; with further model training, we also

anticipate an improvement in the accuracy of the image classifier for identification of MI. Significantly, no false positives for MI were recorded. Future development of the image classifier may ultimately reach sensitivity levels that will limit the number of cases in need of confirmatory stains for myoepithelial markers, leading to TAT improvement and decreased costs.

164 Lymphoma of the Breast: A Single Healthcare System Experience

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Background: Breast lymphoma (BL) is a rare entity that represents 1-2% of breast malignancies while being the most common extra-mammary cancer involving the breast. It occurs either as primary extranodal lymphoma or as secondary involvement by systemic disease. We aimed to report a single health care system experience in BL.

Design: We conducted a review of primary and secondary BL seen at our health care system between 2010 and 2019. Clinical and pathological characteristics were assessed retrospectively. Primary lymphoma was defined as involvement of breast as the first clinical presentation with or without synchronous axillary lymph node involvement, and absence of systemic disease. Fifty-three BL from 47 patients was identified and included in the study. 31 of 53 (58%) BL were categorized as primary and 22 (42%) of them were secondary.

Results: The median age was 71 and 73 years for PBL and SBL. Two patients in each category were male. Nine of 31 (29%) patients with PBL and 6 of 22 (27%) patients with SBL had a history of breast cancer in either breast. Approximately two-thirds of PBL occurred in the right breast whereas SBL effected both breasts equally. Three patients in each category had bilateral breast involvement by lymphoma. Most common lymphoma was diffuse large B cell lymphoma (DLBCL) in PBL category and MALT lymphoma in SBL category (Table 1). One patient had synchronous primary anaplastic large cell lymphoma (ALCL) and secondary follicular lymphoma (FL) in the same breast. Among 6 patients with bilateral BL, 3 patients (1 PBL, 2 SBL) had peripheral T cell lymphoma (PTCL, NOS), 1 patient (PBL) had DLBCL, 1 had (PBL) chronic lymphocytic lymphoma (CLL) and 1 (SBL) had MALT lymphoma. Six (19%) patients with PBL and 1 (5%) with SBL had ipsilateral axillary lymph node involvement. One patient with primary CLL recurred after 8 years and 2 patients with secondary MALT lymphoma recurred after 3 and 7 years.

Characteristics	**Primary (31)**	**Secondary (22)**
Age (years)	**N *(%)***	**N *(%)***
Median (range)	71 (44-91)	73 (23-89)
< 50 years old	3 *(10%)*	1 *(5%)*
≥ 50 years old	28 *(90%)*	21 *(95%)*
Sex		
Female	29 *(94%)*	20 *(91%)*
Male	2 *(6%)*	2 *(9%)*
History of Breast CA		
No	22 *(71%)*	16 *(73%)*
Yes	9 *(29%)*	6 *(27%)*
Side		
Left	12 *(39%)*	11 *(50%)*
Right	19 *(61%)*	11 *(50%)*
Bilateral		
Yes	3 *(10%)*	3 *(14%)*
No	28 *(90%)*	19 *(86%)*
Pathology		
DLBCL	9 *(29%)*	2 *(9%)*
LGBCL (NOS)	6 *(19%)*	3 *(14%)*

MALT lymphoma	4 *(13%)*	6 *(27%)*
CLL	4 *(13%)*	4 *(18%)*
FL	3 *(10%)*	2 *(9%)*
PTCL (NOS)	2 *(6%)*	5 *(23%)*
MCL	1 *(3%)*	0 *(0%)*
HL	1 *(3%)*	0 *(0%)*
ALCL	1 *(3%)*	0 *(0%)*
Axillary LN involvement		
Yes	6 *(19%)*	1 *(5%)*
No	25 *(81%)*	21 *(95%)*
Recurrence		
Yes	1 *(3%)*	2 *(9%)*
No	30 *(97%)*	20 *(91%)*
Abbreviations: DLBCL; diffuse large B cell lymphoma, LGBCL; low grade B cell lymphoma, MALT; mucosa-associated lymphoid tissue, CLL; chronic lymphocytic lymphoma, FL; follicular lymphoma, PTCL; peripheral T cell lymphoma, MZL; mantle cell lymphoma, HL; Hodgkin's lymphoma, ALCL; anaplastic large cell lymphoma, NOS; not otherwise specified		

Conclusions: The most common PBL was DLBCL at our healthcare system; however, the frequency was lower than seen in the previous series. Overall the most common BL and SBL was MALT lymphoma. The significant number of patients (28%) had a history of breast cancer, and ipsilateral axillary LN involvement was observed mainly in patients with PBL in our series. Lastly, compare to the published literature, we report a higher frequency of male patients, PTCL (NOS) and MALT lymphoma, and lower frequency of DLBCL as well as FL.

165 Recurrence Rates of Benign and Borderline Phyllodes Tumors after Surgical Excision

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Disclosures: Iskender Genco: None; Sabina Hajiyeva: None

Background: Local recurrence (LR) has been an important issue for patients with breast phyllodes tumors (PTs). More studies are needed to investigate whether different treatment strategies could be applied to different grades of PTs. In this study, the risk factors for recurrence of benign PT (BePT) and borderline PTs (BoPT) were analyzed.

Design: Our pathology database was searched for breast surgical excision specimens with a diagnosis of BePT and BoPT from 2008 to 2018. Patients with follow-up information at least 6 months after surgery were included in the study. Clinical features for all cases (112) were evaluated. Slide review was performed for 69 cases. Margin status was defined as; positive, ≤ 1 mm and negative. Cases were considered as "recurrence" when a new PT was identified in the same quadrant of ipsilateral breast.

Results: A total of 171 cases (158 BePT, 13 BoPT) were surgically excised in our institution between 2008 and 2018. Of these, 112 cases (105 BePT, 7 BoPT) from 98 patients had follow-up information. 90 patients had one PT, 6 patients had 2 PTs, and 1 had 4 and 1 had 6 PTs. The mean and median age of the patients was 39 and 49 years. Tumors ranged from 5 mm to 69 mm in size with a median size of 12 mm. Surgical margin was positive in 67 (60%) cases, ≤ 1 mm in 21 (19%) cases and negative in 24 (21%) cases. 10 of 21 (48%) cases with ≤ 1 mm margin and 56 of 67 (84%) cases with positive margin were re-excised and had a final negative margin. The final margin status was positive in 11 (10%), ≤ 1 mm in 11 (10%) and negative in 90 (80%) cases (Table1). The median follow-up period was 30 months, ranging from 6 months to 132 months. Three of 112 (2.7%) PTs recurred during follow-up. The time interval to recurrence was 7, 42 and 62 months. All were BePT in original and recurrence site. None of BoPTs recurred during follow-up. There was no statistically significant difference in any clinical or histological features between patients with recurrence and no recurrence.

Characteristics	Recurrence (3)	Non-recurrence (109)
Age (years)	**N *(%)***	**N *(%)***
Median (range)	61 *(42-78)*	65 *(43-98)*
< 50 years old	4 *(17%)*	2 *(9%)*
≥ 50 years old	19 *(83%)*	20 *(91%)*
Size (cm)		
Median (range)	39 *(33-41)*	40 *(20-69)*
Grade		
Benign	3 *(100%)*	102 *(94%)*
Borderline	0 *(0%)*	7 *(6%)*
Border *		
Circumscribed	2 *(67%)*	51 *(77%)*
Infiltrative	1 *(33%)*	15 *(23%)*
Stromal overgrowth *		
Present	0 *(0%)*	5 *(8%)*
Absent	3 *(100%)*	61 *(92%)*
Atypia *		
Mild	2 *(67%)*	51 *(77%)*
Moderate	1 *(33%)*	15 *(23%)*
Marked	0 *(0%)*	0 *(0%)*
Mitotic rate *		
Median (range)	0 *(0-2)*	1 *(0-10)*
< 2	2 *(67%)*	48 *(73%)*
≥ 2	1 *(33%)*	18 *(27%)*
Final margin		
Positive	1 *(33%)*	10 *(9%)*
≤ 1 mm+Negative	2 *(67%)*	99 *(91%)*
* Slide review to document these features was performed in 69 cases. Chi-square and Student t-test analysis revealed no statistically significant clinical or histological risk factor for recurrence (p>0.5)		

Conclusions: We found a very low recurrence rate (2.7%) of overall benign and borderline PTs in our institution. Patients with positive final surgical margin had a higher recurrence rate compared to patients with ≤ 1 mm+negative margin, however; the difference was not statistically significant.

166 High EZH2 Expression in DCIS on Needle Core Biopsy is an Independent Predictive Factor for Upgrade on Surgical Excision

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Background: Approximately 25% of ductal carcinoma in situ (DCIS) diagnosed on breast core biopsy is upgraded to invasive carcinoma on surgical excision. Risks factors to predict upgrade on excision are not well established which leads many patients to be over or under-treated. Enhancer of zest homolog 2 (EZH2) was shown to be associated with aggressive behavior of cancer from many sites, including breast cancer. It was also described in relation to high-risk features in DCIS. We aimed to analyze EZH2 expression in DCIS as a predictor factor for an upgrade on excision.

Design: Our pathology database was searched for DCIS diagnosed on core biopsy. We identified 23 patients who underwent subsequent surgical excision after the diagnosis of DCIS on core biopsy and were upgraded to invasive carcinoma on excision. For the majority of

patients with an upgrade, a control patient with biopsy-proven DCIS who did not show upgrade on excision was identified and matched based on nuclear grade and presence of necrosis. Immunohistochemical stain for EZH2 (11, Cell Marque) was performed on core biopsy specimens and its expression was compared with clinic-pathological features of the patients. EZH2 expression was evaluated in the nuclei of DCIS cells. As described in prior studies, a staining score (range 0-12) was obtained by multiplying the staining intensity (0 - no staining, 1 - weak, 2 - moderate, 3 - strong) and proportion of positive cells (0 - < 1%, 1 – 1-25%, 2 - 25-50%, 3 - 50-75%, 4 - >75%). Nuclear EZH2 expression was considered as 'high expression' if the score was ≥6. Univariate and multivariate logistic regression models were used to compare the variables in SPSS and p value <0.05 was considered as significant.

Results: 25 of 45 (56%) DCIS showed high EZH2 nuclear expression. High EZH2 expression was significantly correlated with ER and PR negativity but not with the other clinico-pathological characteristics (Table 1). When we compared upgraded and non-upgraded cases by using univariate logistic regression analysis, ER and PR negativity, as well as high EZH2 expression were significantly correlated with an upgrade on excision. Multivariate logistic regression analysis revealed high EZH2 expression as the only independent predictor factor for an upgrade on excision.

Characteristics	**Upgrade (23)**	**Non-upgrade (22)**	**Univariate**	**Multivariate**
Age (years)	**N *(%)***	**N *(%)***	***p value***	***p value***
Median (range)	61 *(42-78)*	65 *(43-98)*	*0.296*	-
< 50 years old	4 *(17%)*	2 *(9%)*	*0.723*	-
≥ 50 years old	19 *(83%)*	20 *(91%)*		
Size of the largest involved duct (mm)				
Median (range)	15 (0.5-3)	15 (0.2-5)	*0.839*	-
< 2mm	17 *(74%)*	15 *(68%)*	*0.671*	-
≥ 2mm	6 *(26%)*	7 *(32%)*		
Predominant Pattern				
Solid	17 *(74%)*	13 *(59%)*	*0.292*	-
Cribriform	4 *(17%)*	8 *(36%)*		
Micropapillary	2 *(9%)*	1 *(5%)*		
Nuclear grade				
2	4 *(17%)*	6 *(27%)*	*0.425*	-
3	19 *(83%)*	16 *(73%)*		
Necrosis				
Present	17 *(74%)*	16 *(68%)*	*0.672*	-
Absent	6 *(26%)*	6 *(32%)*		
Microcalcifications				
Yes	13 *(57%)*	15 *(68%)*	*0.420*	-
No	10 *(43%)*	7 *(32%)*		
ER receptor				
Positive	11 *(48%)*	18 *(82%)*	*0.017*	*0.496*
Negative	12 *(52%)*	4 *(18%)*		
PR receptor				
Positive	8 *(35%)*	16 *(73%)*	*0.011*	*0.871*
Negative	15 *(65%)*	6 *(27%)*		
EZH2				
High	19 *(83%)*	6 *(27%)*	*<0.001*	***0,004****
Low	4 *(17%)*	16 *(73%)*		

** Multivariate logistic regression analysis by including ER receptor, PR receptor and EZH2 expression as covariates revealed high EZH2 expression as the only independent predictor factor for an upgrade on excision.*

Conclusions: We found that high nuclear EZH2 expression is an independent predictor factor for an upgrade on excision. More studies are needed to explore the role of EZH2 expression in DCIS for diagnostic, treatment and prognostic purposes.

167 Concordance in Breast Cancer Grading on Digitally Scanned Slides: A Multi-Institutional Study

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Background: Histologic grading is an important prognostic feature in breast carcinoma evaluation which has been incorporated into the 8th edition of the AJCC Cancer Staging Manual. For prognostic markers such as grade to be robust, there must be high reproducibility and low interobserver variability. We sought to evaluate interobserver variability amongst a multi-institutional group of academic breast pathologists using whole slide scanned images.

Design: 150 consecutive invasive breast carcinomas were identified from a single participating institution. A representative slide was selected and scanned into a digital slide platform. The scanned slides were independently reviewed by 6 pathologists and assigned grades (1, 2, or 3) based on established criteria for tubule formation (TF), nuclear pleomorphism (NP), and mitotic count (MC). Fleiss' k for overall agreement amongst all observers was calculated for overall grade and individual components. The most common grade (statistical mode) was taken as the gold standard, and interobserver concordance was evaluated based on this grade, as well as histopathologic type.

Results: All patients were female with a mean age of 63 years (range; 29-98). 113 cases (75.3%) were invasive ductal (no special type), 25 (16.7%) were invasive lobular, and 12 (8.0%) were other special types. Overall, interobserver agreement for grade was moderate (k=0.501), with the best agreement amongst grade 1 (k=0.694), followed by grade 3 (k=0.503), and only fair agreement for grade 2 (k=0.375). Perfect agreement was observed in 46 cases. 4 cases demonstrated a two-step discrepancy, 3 of which also showed two-step discrepancy in mitotic rate. In 14 cases, there was an even split between pathologists in terms of grade. Excluding the 14 cases with an even split between grades, complete concordance amongst all pathologists was observed in 59%, 21%, and 38% of tumors with a modal grade 1, 2, and 3, respectively (p=0.006). Interobserver agreement was fair to moderate for the individual components (Table 1). Interobserver agreement was better for patients with invasive ductal (k=0.501) than invasive lobular carcinomas (k=0.104).

Table 1: Interobserver Variability of Individual Grading Components

Component	Fleiss' k
Tubule Formation	0.495
Nuclear pleomorphism	0.401
Mitotic Rate	0.288

Conclusions: Pathologists have reasonable (but imperfect) agreement in grading breast carcinomas on digitally scanned slides, particularly those that are ductal in histology and/or at the extremes of grade. How the residual variability affects stage and treatment decisions as well as the impact of evaluating mitotic rate on digitized images remains to be elucidated.

168 Correlation Between Modified Magee Equation-2 and Oncotype-Dx Recurrence Scores and its Clinical Application: A Single Institutional Review

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Disclosures: Akisha Glasgow: None; Haley Sechrist: None; Philip Bomeisl: *Consultant*, PathAI; Hannah Gilmore: None; Aparna Harbhajanka: None

Background: Oncotype Dx recurrence score (ODX-RS) is used to predict recurrence in breast cancer, however, recent studies show that the modified Magee equations (MME) provides an equitable estimate of the actual ODX-RS in distinct cohorts. The aim of this study is to compare ODX-RS and the MME-RS using TAILORx cutoffs and to apply and assess the correlation of the recently proposed algorithmic approach to distinct cohort using MME-2.

Design: All cases of newly diagnosed breast cancer were identified with available ODX RS and the MME-2 was calculated. Cases were stratified in distinct cohorts based on the recently described algorithmic approach with different combinations of MME-2 RS and ODX-RS to assess correlation and to find the cohort in which ODX testing can be safely omitted.

Results: A total of 579 patients were identified in our cohort from 2012-2017. There was significant correlation between MME-2 and ODX-RS (Pearson correlation coefficient=0.635 (p<0.0001)). The overall agreement between ODX and MME-2 using traditional cutoffs was

64.4% (373/579) with agreements of 71.4%, 52.2% and 84.2% for low, intermediate and high-risk categories, respectively (Table 1). Using TAILORx cutoffs, the overall agreement was 64.2% (372/579) with agreements of 28.9%, 66.4%, and 74.1% for low, intermediate and high-risk categories, respectively (Table 1). When the patients were further stratified based on the known algorithm, the correlation between the cohort of MME-2 RS with cutoff 18 and ODX-RS<25 was 70.3% (407/579). In addition, using <25 as the ODX-RS cutoff and MME-2 RS<18 there was a correlation of 96.1% (323/336) and when the cohort of MME-2 RS >30 compared with ODX-RS>25, the correlation was 89.5% (17/19). However, the correlation was 94.4% (238/252) when patients were further stratified based on mitotic score, MME-2 RS 18-25 and mitotic score of 1 and compared with ODX-RS<25.

Part A.				
Cohort based on Traditional Cutoffs MME-2-RS and ODX-RS				
	Oncotype			
MME-2 RS	<18	18-30	>30	Total
<18	240 (71.4%)	92 (27.4%)	4 (1.2%)	336 (100%)
18-30	74 (33%)	117 (52.2%)	33 (14.7%)	224 (100%)
>30	1 (5.3%)	2 (10.5%)	16 (84.2%)	19 (100%)
Total	315 (54.4%)	211 (36.4%)	53 (9.2%)	579 (100%)
Cohort based on TAILORx Cutoffs MME-2-RS and ODX-RS				
	Oncotype			
MME-2 RS	<11	11-25	>25	Total
<11	13 (28.9%)	30 (66.7%)	2 (4.4%)	45 (100%)
11-25	110 (23.1%)	316 (66.4%)	50 (10.5%)	476 (100%)
>25	1 (1.7%)	14 (24.1%)	43 (74.1%)	58 (100%)
Total	124 (21.4%)	360 (62.2%)	95 (16.4%)	579 (100%)
Cohort based on MME-2 Cutoff 18 and ODX Cutoff 25				
		Oncotype		
<18		<25	>25	Total
MME-2	<18	323 (96.1%)	13 (3.9%)	336 (100%)
	>18	159 (65.4%)	84 (34.6%)	243 (100%)
	Total	482 (83.2%)	97 (16.8%)	579 (100%)
p<0.0001				
Cohort further stratified based on mitotic score in MME-2 Cutoff 18-25 and ODX Cutoff 25				
MME-2 18-25		Oncotype		
	Mitotic Count	<25	>25	Total
	1	238 (94.4%)	14 (5.6%)	252 (100%)
	2	52 (71.2%)	21 (28.8%)	73 (100%)
	3	15 (65.2%)	8 (34.8%)	23 (100%)
	Total	305 (87.6%)	43 (12.4%)	348 (100%)
p<0.0001				
Cohort based on MME-2 Cutoff 30 and ODX Cutoff 25				
MME-2>30		Oncotype		
		<25	>25	Total
MME-2 RS	<30	480 (85.7%)	80 (14.3%)	560 (100%)
	>30	2 (10.5%)	17 (89.5%)	19 (100%)
	Total	482 (83.2%)	97 (16.8%)	579 (100%)
p<0.0001				

Conclusions: There is a significant correlation between MME-2 and ODX-RS. The overall agreement between MME-2 and ODX-RS were similar for both TAILORx and traditional cutoffs. The highest correlation was identified between the cohort of MME-2 RS >30 compared with ODX-RS>25 (89.5%). However, when further stratified based on mitotic score, the cohort of MME-2 RS 18-25 with mitotic score of 1 and ODX-RS<25 showed the highest correlation (94.4%). Further studies are required to confirm the clinical application of MME-2.

169 Molecular Pathways Associated with Recurrence of Ductal Carcinoma In Situ in Singaporeans

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Background: Ductal carcinoma in situ, the most common type of pre-invasive lesion, is being detected with increasing frequency with the advent of mammographic screening. Race/ethnicity is an emerging factor in the risk of DCIS recurrence or progression to invasive breast

cancer (IBC). In particular, an increased risk of recurrence has been observed in Asian women with DCIS. The underlying biology and molecular markers that drive the recurrence of DCIS associated in Asian populations need to be elucidated.

Design: Transcriptomic profiling (Human Clariom D Assay) was performed in de-identified 74 ductal carcinoma *in situ* (DCIS) cases obtained from the archives at Singapore General Hospital. Of the 67 cases that passed quality control, 39 cases were mastectomies (28 ERpos; 11 ERneg; 19 recurrences). 16 ERpos cases recurred after mastectomy (MR) and 12 cases did not recur (MNR). Similarly there were 3MR and 8MNR in the 11 ERneg cases. Clinical information on the samples included age at diagnosis, size (mm) of ductal carcinoma in situ and follow-up for recurrence. Principal component analysis (PCA), ANOVA, and pathway analysis were performed to identify genes and pathways associated with recurrences.

Results: The PCA showed considerable degree of overlap in the MR versus MNR analysis, however the cases were better distinguished when ERpos and ERneg cases were separately analyzed. Comparison of MR versus MNR in ERpos and ERneg subgroups revealed 80 genes were significantly altered in both groups, whereas 39 and 194 genes were unique to ERpos and ERneg, respectively ($P<0.05$; -22). The DAVID Functional Annotation Clustering Analysis showed enrichment of ribosome, RNA binding and cell adhesion networks in recurrent (ERpos and ERneg) cases; additionally ERneg recurrent cases were also enriched of mitochondrial electron transport pathways.

Conclusions: This study, to our knowledge, provides the first comprehensive transcriptomic analysis of pure DCIS cases with recurrence that characterizes the underlying biological networks in a cohort of Singapore Asian women being the majority from the Chinese ethnicity. As these patients had undergone mastectomy, the impact of other treatment parameters such as margin status, radiation did not impact our data providing a clearer picture of transcriptomic features associated with recurrence. Validation of these markers will determine their diagnostic and therapeutic utility.

170 Digital Droplet PCR (ddPCR) for HER2 Amplification in Breast Cancer

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Background: Although it has been two decades since the first HER2-targeted therapy became available, standardizing HER2 testing and defining HER2 amplification have been challenging. ASCO/CAP guidelines were revised in 2018 to create five algorithmic groups. While groups 1 and 5 represent unambiguous amplified and non-amplified cases, groups 2-4 require time consuming and costly additional testing and constitute >20% of all HER2 FISH testing in the reference lab setting. ddPCR has many advantages over traditional FISH and IHC testing, including its highly quantitative, inexpensive, rapid, and far less labor-intensive methodology. It has been shown to be sensitive and specific for breast cancer HER2 testing and to have good concordance with traditional methods. We now evaluate HER2 ddPCR in group 2-4 equivocal cases by 2018 guidelines compared to FISH/IHC and assess clinical outcome.

Design: We designed a 2-well multi-amplitude ddPCR assay to test for amplification of three HER2 regions against three reference locations. FFPE breast tissue from non-neoplastic controls (N=16) determined the normal distribution of HER2/Reference ratios by ddPCR. Group 5 (N=10) and group 1 (N=20) cases by 2018 guidelines were used to determine sensitivity and specificity of the ddPCR assay. Using these defined cutoffs, 30 group 2-4 equivocal cases were tested by ddPCR. Results were compared to FISH/IHC and response to HER2-targeted treatment.

Results: HER2 ddPCR amplification sensitivity was 95% and specificity 90%, showing high concordance with FISH in groups 1 and 5. Equivocal groups 2-4 by FISH/IHC agreed with ddPCR in only 16/30 cases (53%) and 13 were reclassified to amplified by ddPCR. Considering only the equivocal group 2-4 patients, 12 received treatment including HER2-targeted therapy with 11 clinical responses (mean follow-up 47 mon; SD 30 mon). Only 2 of the 11 (18%) responders would have qualified for HER2 therapy under 2018 guidelines, whereas 8 (73%) would have qualified by ddPCR.

Conclusions: In conclusion, ddPCR is a robust method for HER2 amplification in FFPE breast cancer. It reveals strong concordance with traditional FISH testing in unambiguous cases. Preliminary results for HER2 amplification by quantitative ddPCR in FISH/IHC equivocal cases show significant discordance (53%). ddPCR compared to FISH/IHC yielded improved prediction of response (73% vs 18%) to treatment regimens that include HER2-targeted therapy.

171 Presence of Internal Controls in Hormone Receptor Testing is Dependent on the Tumor Subtype and Should be Considered in ASCO/CAP Guideline Revision

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Disclosures: Eric Goold: None; Jolanta Jedrzkiewicz: None; H. Evin Gulbahce: None

Background: It is recommended that estrogen (ER) and progesterone (PR) receptor status be determined on all newly diagnosed breast cancers. ASCO/CAP guidelines control pre-analytical, analytical and post-analytical variables to decrease erroneous results especially false negative testing. The upcoming revision emphasizes presence and staining of internal controls (IC) as another step in quality assurance. We have noticed that many hormone receptor negative cancers do not have an IC in diagnostic biopsies-when we needed it the most. The purpose of this study is to determine if there is a correlation between the hormone receptor status of tumor and likelihood of finding IC in the test tissue-which will be critical in assuring a negative test is valid.

Design: All ER and PR tests on breast cancer submitted to our national reference laboratory between 1/2014-4/2019 were identified. Our lab reports intensity and percent staining for cancer and presence/absence of IC for ER and PR separately. Cases signed out by one attending who doesn't report presence of IC when the carcinoma is hormone receptor positive were excluded. Age adjusted logistic regression was performed to determine factors associated with presence of IC.

Results: 663 breast cancer samples were submitted for ER/PR testing in which the presence/absence of IC were recorded. 153 (23.1%) of the cancers were ER negative (<1%), 31 (4.7%) had 1-10% ER expression, 479 (72.2%) showed > 10% ER expression. 253 of the cancers (38.2%) were PR negative (<1%), 51 (7.7%) had 1-10% PR expression, 358 (54.1%) showed > 10% PR expression. In one, no tumor was present in PR slide. 551 (82.3%) of the cases were ER and/or PR positive whereas 112 (17.7%) were negative for both ER and PR. We found about 50% decrease in probability of finding IC for ER and PR in hormone negative cancers (Table 1). Compared to high (≥90) hormone expressors, which is the majority of tumors, the likelihood of hormone receptor negative tumors having IC for ER and PR was significantly decreased (p<.001) (Table 1).

Probability for Having Internal Control for *						
	ER			PR		
	OR	CI	*p*	OR	CI	*p*
Hormone Receptor Negative (ER-/PR-) Cancers (n=112)	0.53	(0.36, 0.88)	< .001	0.46	(0.32, 0.67)	< .001
ER in Cancer (%)						
≥90	REF			REF		
>50-<90	0.47	(0.19, 1.17)		0.80	(0.45, 1.42)	
11-50	0.64	(0.27, 1.51)		0.73	(0.42, 1.28)	
1-10	0.66	(0.33, 1.33)		0.60	(0.31, 1.18)	
<1	0.47	(0.32, 0.70)	< .001	0.45	(0.30, 0.69)	< .001
PR in Cancer (%)						
≥90	REF			REF		
>50-<90	0.48	(0.19, 1.20)		0.75	(0.42, 1.34)	
11-50	0.55	(0.24, 1.28)		0.66	(0.38, 1.14)	
1-10	0.61	(0.31, 1.21)		0.57	(0.29, 1.11)	
<1	0.43	(0.29, 0.63)	< .001	0.36	(0.23, 0.55)	< .001
* adjusted for age. OR: odds ratio; CI: confidence interval						

Conclusions: Presence of IC for ER/PR in the tested tissue is dependent on the tumor subtype, is 50% less likely in hormone negative cancers, and should be taken into consideration when revising ASCO/CAP guidelines. Larger size of hormone negative tumors leaving no

normal epithelium and percolating nature of some hormone positive tumors with “trapped” normal ducts/acini may be contributing factors to this difference.

172 Estrogen Receptor Positive Advanced Breast Cancer Harbors Frequent GATA3 Mutations

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Background: GATA3 is an essential transcription factor which is linked to the ER signalling pathway in breast cancer. Genomic alterations (GAs) in *GATA3* gene have recently been described in approximately 10% of breast tumors. The aim of this study was to identify breast tumors with *GATA3* GAs and delineate clinicopathologic and molecular features of *GATA3* mutated (*GATA3*mut) tumors.

Design: Thirty six samples of advanced stage, treatment resistant breast carcinoma were assayed by hybrid capture-based comprehensive genomic profiling (CGP) including 324 cancer-related genes and introns from 28 genes frequently rearranged in cancer. GAs were correlated with clinicopathologic data. IHC for GATA3 (clone L50-823, Biocare Medical) was performed in all *GATA3*mut cases.

Results: Nine (25%) breast carcinomas featured *GATA3* genomic alterations (Table). Six *GATA*3mut samples used for CGP were metastatic biopsies and 3 were primary breast tumors. The majority of GAs (7/9) were frameshift mutations, one case harbored a missense mutation, and another had a nonsense mutation. Three *GATA3* mutations were in the second zinc finger (ZnFn2) domain, responsible for DNA binding, two mutations were between ZnFn1 and ZnFn2 domains, and the rest were towards the C-terminus. Interestingly, all *GATA3*mut cases were positive for GATA3 by IHC. All *GATA3*mut cases were ER+ and 2 of them (22%) were HER2+. Of the 27 *GATA3* wild type (*GATA3*WT) cases, 15 were ER+ and one of the 15 (6.6%) was HER2+. *GATA3* GAs were present in 38% of all ER+ tumor cases (24 in total). The *GATA3*mut group had fewer GAs per tumor as compared to the *GATA3*WT ER+ group (6.1 versus 8.7). The most common GAs in both groups were *PIK3CA*, *CCND1, FGF3, FGF4,* and *FGF19* with similar frequencies. In contrast, *TP53* mutations were disproportionally associated with *GATA3*WT ER+ group (53.3%) as opposed to 11.1% in *GATA3*mut group ($p = 0.038$). Similarly, 33% of *GATA3*WT ER+ tumors featured *MYC* GAs, while no (0%) *MYC* GAs were present in *GATA3*mut group. In addition to potentially targetable *PIK3CA* GAs, rare targetable mutations were identified in *ESR1* gene, with higher frequency in *GATA3*WT ER+ group.

	GATA3 mutated tumors	*GATA3* wild type	*GATA3* wild type ER+
Number of cases	9	27	15
Median Age (Range) in years	60 (30-78)	58 (41-78)	56 (41-73)
GAs/tumor	6.1	8	8.7
Targetable GAs/tumor	1.3	2.2	2.2
Hormone receptor status			
ER+, HER-2-	77.7% (7)	52% (13/25)	86.6% (13)
ER+, HER-2+	22% (2)	4% (1/25)	6.6% (1)
ER-, HER-2+	0% (0)	4% (1/25)	0% (0)
ER-, HER-2-	0% (0)	24% (6/25)	0% (0)
Most frequently altered genes as % of cases affected (# of cases affected)	33.3% (3): *CCND1*, *FGF3*, *FGF4*, *FGF19*, *PIK3CA*	62.9% (17): *TP53*	53.3% (8): *TP53*
	22.2% (2): *ARID1A*, *FOXP1*, *IGF1R*	37% (10): *MYC*	40% (6): *CCND1*, *FGF3*, *FGF4*
	11.1% (1): *TP53*	29.6% (8): *CCND1*, *FGF4*, *PIK3CA*	33.3% (5): *FGF19*, *MYC*, *PIK3CA*
		25.9% (7): *FGF3*, *FGF19*	26.6% (4): *MAP3K1*
		18.5% (5): *CDH1*, *PTEN*	20% (3): *CDH1*, *ESR1*, *FGFR1*, *PTEN*, *ZNF703*
		14.8% (4): *FGFR1*, *MAP3K1*, *MCL1*	13.3% (2): *AURKA*, *CCND3*, *CREBBP*, *FRS2*, *GNAS*, *IGF1R*, *KDM5A*, *MYST3*, *TERC*, *ZNF217*
		11.1% (3): *AKT1*, *AURKA*, *BRCA1*, *CCND3*, *CREBBP*, *ESR1*, *FRS2*, *GNAS*, *IGF1R*, *KRAS*	
Potential Targeted Therapy Impacting			

Alterations 100*GAs/cases (#GAs)			
	44.4 (4) *PIK3CA*	37 (10): *PIK3CA*	46.6 (7): *PIK3CA*
	11.1 (1): *ESR1*	11.1 (3): *ESR1*	20 (3): *ESR1*

Conclusions: *GATA3* mutations are present in significant proportion of advanced breast cancer. All *GATA3*mut cases are ER+, and in some cases may be HER2+. The fact that all *GATA3*mut cases were immunolabelled by a commonly used GATA3 antibody argues against an IHC approach to exclude a *GATA3*mut. *TP53* GAs are rare among the *GATA3mut* cases and GAs in *GATA3* and *MYC* are mutually exclusive.

173 PD-L1 Expression in Metaplastic Breast Carcinoma (MBC) Using PD-L1 SP142 Assay and Concordance Among PD-L1 Immunohistochemical (IHC) Assays

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Background: MBC is an aggressive, usually triple negative (TN), histologic subtype. Previous study of PD-L1 in MBC scored tumor cells (TC), different from the current scoring method. In this study, we assessed PD-L1 expression in MBC using the FDA-approved SP142 assay to evaluate the potential eligibility of MBC for recently FDA-approved anti-PD-L1 therapy. We also evaluated the concordance of two IHC assays.

Design: Primary, treatment-naïve MBC treated at our Center from 1998-2019 were identified. Clinicopathologic features were reviewed. PD-L1 expression was assessed using SP142 (Ventana) and novel anti-PD-L1 mAb 73-10 (Abcam). PD-L1 scoring followed criteria in the IMpassion130 trial: PD-L1 expression on tumor infiltrating immune cells (IC) as a percentage of tumor area (<1% negative vs ≥1% positive).

Results: A total of 44 MBCs were identified. Histologic subtypes included: 48% (21/44) matrix-producing (MP), 34% (15/44) spindle cell (SP) and 18% (8/44) squamous cell (SCC). The majority (81%) were TN (Table 1). Using SP142, PD-L1 IC positivity was seen in 91% (40/44) MBCs, with similar rates in MBCs with MP (90%, 19/21), SP (87%, 13/15), and SCC (100%, 8/8). We also observed PD-L1 expression ≥1% in TC in 23% (10/44) MBCs: 19% (4/21) MP, 30% (5/15) SP, and 13% (1/8) SCC. Using 73-10, 93% (41/44) MBC IC were positive. The 73-10 assay showed more positivity in TC than SP142 (43% vs 23%, p=0.67). The overall concordance for IC scoring between the assays was 93%. Discrepant scoring on IC was seen in 7% (3/44) of cases: 2 negative with SP142 were positive with 73-10, and 1 positive with SP142 was negative with 73-10. Discrepant scoring on TC was seen in 20% (9/44) of cases: 9 negative by SP142 were positive with 73-10. The SP142 assay showed a distinct difference in staining between IC (granular, dot-like) and TC (membranous) while, 73-10 showed membranous +/- cytoplasmic staining in both IC and TC.

Age (years)	Tumor size (cm)	Histologic subtype	ER	HER2	PD-L1 SP142 IC	PD-L1 73-10 IC	PD-L1 SP142 TC	PD-L1 73-10 TC
65	1.1	MP	Neg	Neg	Pos	Pos	Neg	Pos
48	3.0	MP	Neg	Neg	Pos	Pos	Neg	Neg
49	1.4	MP	Neg	Neg	Pos	Pos	Neg	Neg
57	1.6	MP	Neg	Neg	Pos	Pos	Pos	Pos
60	2.1	MP	Neg	Neg	Pos	Pos	Neg	Neg
59	1.2	MP	Neg	Neg	Pos	Pos	Neg	Neg
75	3.0	MP	Neg	Neg	Pos	Pos	Neg	Neg
85	3.9	MP	Neg	Neg	Pos	Pos	Neg	Pos
63	0.9	MP	Neg	Neg	Pos	Pos	Neg	Neg
52	1.5	MP	Neg	Neg	Pos	Pos	Pos	Pos
82	1.8	MP	Neg	Neg	Pos	Pos	Neg	Neg
57	1.1	MP	Neg	Neg	Neg	Pos	Neg	Neg
58	1.0	MP	Pos	Neg	Neg	Neg	Neg	Pos
75	3.7	MP	Neg	Neg	Neg	Pos	Neg	Neg
65	1.5	MP	Neg	Neg	Pos	Pos	Neg	Neg
37	2.2	MP	Neg	Neg	Pos	Pos	Pos	Pos
63	2.7	MP	Neg	Neg	Pos	Pos	Pos	Pos
52	1.3	MP	Neg	Neg	Pos	Neg	Neg	Neg
50	1.4	MP	Neg	Neg	Pos	Neg	Neg	Neg
39	1.7	MP	Neg	Neg	Pos	Neg	Neg	Neg
71	2.2	MP	Pos	Neg	Pos	Neg	Neg	Neg
54	2.5	SP	Neg	Neg	Pos	Pos	Neg	Neg
65	4.4	SP	Neg	Neg	Pos	Pos	Neg	Neg
41	3.9	SP	Neg	Neg	Pos	Pos	Pos	Pos
45	4.6	SP	Neg	Neg	Neg	Pos	Neg	Neg
67	1.0	SP	Neg	Neg	Pos	Pos	Neg	Neg
83	5.9	SP	Neg	Neg	Pos	Pos	Pos	Pos
85	2.5	SP	Neg	Neg	Pos	Pos	Neg	Pos
79	1.5	SP	Neg	Neg	Pos	Pos	Neg	Pos
50	1.0	SP	Neg	Neg	Pos	Pos	Neg	Neg
34	2.9	SP	Neg	Neg	Pos	Pos	Pos	Pos
67	3.5	SP	Neg	Neg	Pos	Pos	Neg	Neg
51	2.5	SP	Neg	Neg	Pos	Neg	Pos	Pos
41	8.0	SP	Neg	Pos	Pos	Neg	Pos	Pos
37	2.4	SP	Neg	Neg	Pos	Pos	Neg	Neg
51	4.0	SP	ND	ND	Neg	Neg	Neg	Neg
72	2.5	SCC	Neg	Neg	Pos	Pos	Neg	Pos
40	2.2	SCC	Pos	Neg	Pos	Pos	Neg	Pos
62	1.3	SCC	Neg	Neg	Pos	Pos	Neg	Pos
78	1.0	SCC	Neg	Neg	Pos	Pos	Pos	Pos
70	3.2	SCC	Neg	Neg	Pos	Pos	Neg	Neg
41	19.0	SCC	Neg	Pos	Pos	Neg	Neg	Pos
59	1.6	SCC	Pos	Neg	Pos	Pos	Neg	Neg
66	14.0	SCC	Neg	Neg	Pos	Neg	Neg	Neg

Table 1: Clinicopathologic Features of Study Cohort. MP – matrix-producing carcinoma, SP – spindle cell carcinoma, SCC – squamous cell carcinoma, IC – immune cells, TC – tumor cells, Neg – Negative, Pos – Positive, ND – not done

Conclusions: The majority of MBC in our cohort were positive for PD-L1 (≥1% IC staining) with both assays (91% vs 93%). This suggests that anti-PD-L1 therapy could be advantageous in MBC. TCs displayed more positive staining on the 73-10 assay. No criteria exist for TC staining and the significance of TC staining in breast cancer has not been fully elucidated. Despite good concordance, the staining patterns were less distinctive using the 73-10 assay. Further study on correlation of PD-L1 positivity with the mutational profiles and clinical outcomes of this aggressive breast cancer subtype is ongoing.

174 Morphologic and Immunohistochemical (IHC) Features of Carcinoma Involving Microglandular Adenosis (MGA) of the Breast Following Neoadjuvant Therapy (NAT)

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Disclosures: Anne Grabenstetter: None; Timothy D'Alfonso: None; Hannah Wen: None; Edi Brogi: None; Lee Tan: None

Background: MGA is a benign breast lesion with infiltrative growth that lacks a myoepithelial cell layer. MGA can exhibit atypia (AMGA) or be involved by carcinoma (CMGA). Since CMGA does not express hormone receptors or HER2 it can be misdiagnosed as invasive triple negative breast cancer (TNBC) on core needle biopsy (CNB) and thus would be more likely to be selected for treatment with NAT. This study examines the morphologic and IHC features of CMGA following NAT.

Design: Post-NAT excisions (EXC) showing AMGA and/or CMGA were identified following a search of our pathology database. Only cases with slides of both the EXC and corresponding CNB available for review were included. Clinicopathologic features and all available slides were reviewed.

Results: Eleven cases from 11 women were identified which met the above criteria. The pathologic features of the cohort are summarized in Table 1. All 11 CNBs were initially diagnosed as invasive ductal carcinoma (IDC); MGA was originally identified in 1 CNB. On morphologic review, 8 CNBs were confirmed to contain AMGA/CMGA, 2 had no evidence of and 1 showed no definite AMGA/CMGA. Definite IDC could not be confirmed in 4 CNBs. At EXC, all 11 cases showed AMGA/CMGA. The 4 cases with no IDC on CNB showed no IDC at EXC and all had persistent AMGA/CMGA. No residual IDC was present in 4 of 7 CNBs with IDC; residual AMGA/CMGA was identified in all 7 EXC. Where IHC was performed on CNB and/or EXC, AMGA/CMGA were positive for S-100, laminin and collagen IV and were negative for calponin and p63. Areas of IDC did not stain with laminin and collagen IV. Following NAT, AMGA/CMGA acquired morphologic alterations that included nuclear pleomorphism, prominent eosinophilic cytoplasmic granules, reduced size of glands, and 6 EXC showed areas of single cell growth pattern which still demonstrated typical staining of MGA (Figure 1).

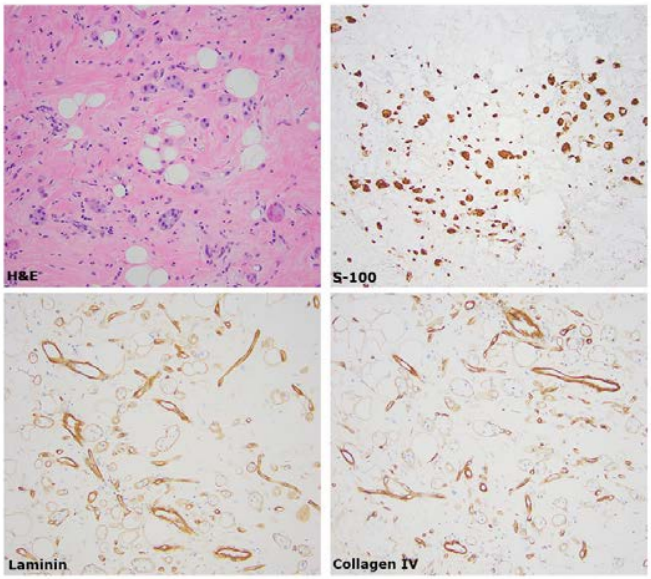
Case	Original Biopsy Diagnosis	Biopsy Morphologic Review			Post-NAT Excision Morphologic Review			Immunohistochemical Findings in AMGA/CMGA		
		IDC	DCIS	AMGA +/- CMGA	IDC	DCIS	AMGA +/- CMGA	S-100	laminin/ collagen IV	calponin/ p63
1	IDC and DCIS	No definite	No	Yes	No	No	Yes	+	+/+	ND
2	IDC	No definite	No	Yes	No	No	Yes	+	+/+	-/-
3§	IDC	No definite	No	Yes	No definite	No	Yes	ND	+/+	ND/-
4	IDC	No	No	Yes	No	Yes	Yes	+	+/+	-/-
5	IDC with metaplastic features in association with AMGA	Yes	No	Yes	Yes (< 2 mm)	No	Yes	+	+/+	ND
6	IDC	Yes	No	Yes	Yes (3 cm)	No	Yes	ND	ND	ND
7	IDC with metaplastic features and DCIS	Yes	No	Yes	No	No	Yes	+	+/+	ND
8	IDC	Yes	No	Yes	No definite	No	Yes	+	+/+	-/ND
9	IDC	Yes	Yes	No*	Yes (6.5 cm)	No	Yes	+	ND	ND
10	IDC and DCIS	Yes	Yes	No*	No	No	Yes	+	ND	ND
11	IDC and DCIS	Yes	Yes	No definite	No	Yes	Yes	+	+/+	ND

Table 1: Pathologic Features of Study Cohort. IDC – invasive ductal carcinoma; DCIS – ductal carcinoma in situ; AMGA – atypical microglandular adenosis; CMGA – carcinoma involving microglandular adenosis; NAT – neoadjuvant therapy; ND – not done

*Consultation slides; MGA not identified in submitted material

§Patient treated with anastrozole only

Figure 1 - 174



Conclusions: This study is the first to examine the effects of NAT on CMGA. Our data showed no response of the CMGA after NAT in contrast to the high response rate of conventional TNBC to NAT. Notably, the infiltrative single cell pattern of residual CMGA could be misdiagnosed for residual IDC. The persistence of CMGA following NAT supports the theory that CMGA is not equivalent to TNBC despite its lack of myoepithelial cells. Our findings also highlighted the challenges in recognizing CMGA in CNBs, and its triple negative phenotype may lead to unwarranted treatment with NAT in the absence of conventional TNBC.

175 An Evaluation of the Concordance of Hormone Receptors (ER and PR) and HER-2 Performed on Breast Core Biopsies and Concurrent Axillary Lymph Node Metastases: A Single Institution Experience

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Disclosures: Mihir Gudi: None; Yen Yeo: None; Unnikrishnan Kuttiparambil: None

Background: Hormone receptor (ER and PR) and HER-2 status of breast carcinoma forms the cornerstone for the management of breast carcinoma as it has both prognostic and therapeutic implications in patient care both in the adjuvant and metastatic setting.

In our institution we currently routinely perform concurrent testing for ER, PgR and HER-2 on every newly diagnosed pre-adjuvant primary breast cancer and the synchronous axillary lymph node metastases as many studies have shown the hormone receptor and HER-2 status may change throughout tumour progression from the primary tumour to the synchronous axillary metastases.

The aim of our study was to compare the hormone receptor profiles and HER-2 status of primary invasive breast carcinoma in the breast core biopsy with the concurrent metastases within ipsilateral axillary lymph node core biopsy. If we can demonstrate high concordance; this would justify performing the immunohistochemistry (IHC) on the breast core biopsy only thus reducing the cost for the patient as well as the laboratory.

Design: We had a total of 1140 cases of invasive breast carcinoma diagnosed in our institution with concurrent ipsilateral axillary lymph node metastases over a period of 4 years (2015-2018). ER and PR were scored using the recommended ASCO/CAP guidelines from 2010 and HER-2 from 2013. For all HER-2 cases if the result was equivocal on either specimen the HER-2 FISH result was taken for final comparison.

Results: RESULTS:

- ER: 2 discordant cases (99.8 % concordance)

- PR: 6 discordant cases (99.4 % concordance)

- Her2: 5 discordant cases (99.5% discordance)

	Breast Biopsy	Axillary lymph Node biopsy	Concordance rate
ER Positive cases	1065 (93.4%)	73 (93.5 %).	99.8%
PR Positive cases	1036 (90.8%)	1031 (90.43%).	99.4%
HER-2 Positive cases	973 (85.3%)	970 (80%)	99.5%

Table 1: Concordance rate for ER, PR and HER-2 on the breast core biopsy and concurrent lymph node metastases.

Figure 1 - 175

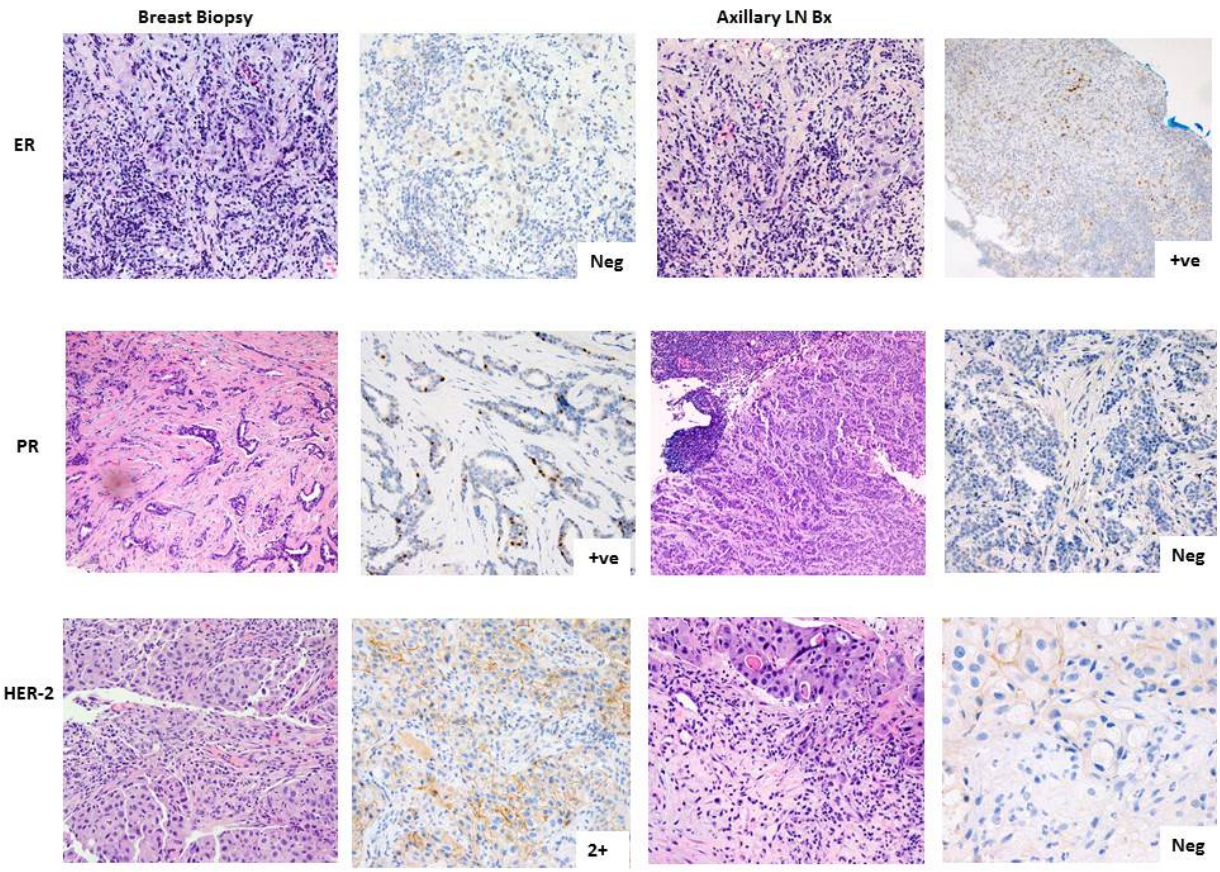

Fig 1. Representative Images of 3 Discordant Cases

Conclusions: The results of the study revealed that there was very good concordance between the results of hormone receptor and HER-2 status performed on the breast core biopsy and the synchronous ipsilateral axillary lymph node metastases. This high concordance rates differs from other studies in the literature where the concordance rates, ranged from 80 -85% for ER, 70 – 85% for PR[1,3,4,5] In contrast most studies have demonstrated a high HER-2 concordance rates[3] of more than 90%. Our results compare well with the study by Zhao S et all [2] with good concordance between the breast core biopsy and axillary lymph node biopsy.

Based on these observations it may be justified not to repeat the hormone receptors and HER-2 stains on the primary beast core and axillary lymph node metastases. This would amount to cost savings both for the patients who in many institutions pay IHC performed per b

176 Expression of Yes-Associated Protein (YAP) in Breast Cancer is Associated with Cancer Stem Cell Markers and its Inhibition Improves the Response to Hormonal Therapy

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Disclosures: Maha Guimei: None; Sana Alrouh: None; Maha Saber-Ayad: None; Yazan Wardeh: None; Tala Bakkour: None; Ahmed El Serafi: None

Background: Yes-associated protein (YAP) is a transcriptional downstream regulatory target in the Hippo signaling pathway. This pathway plays an important role in tumor suppression by restricting proliferation and promoting apoptosis. Activation of YAP results in its translocation to the nucleus thereby promoting a carcinogenic effect. The role of YAP, in breast cancer has been very controversial. The present study aimed to investigate the expression of YAP in different molecular subtypes of breast cancer and its association with expression of cancer stem cells (CSCs) in the tumors.

Design: YAP mRNA and protein expression was characterized in 23 cases of breast carcinomas and 5 normal breast tissue, using RT-PCR and immunohistochemistry. The study also investigated the expression of stem cell markers (SOX-2, NANOG and OCT4). YAP expression was characterized in breast cancer cell lines and the growth and viability of MCF-7 breast cancer cells was studied using MTT assay with and without the YAP inhibitor Verteporfin.

Results: In this study, we report that YAP mRNA expression is significantly higher in breast cancer tissue compared to normal (P=0.040). A statistically significant difference in YAP expression was also found between Luminal B and TNBC (P=0.029). mRNA expression levels of OCT4 and NANOG were significantly increased in breast cancer. Both OCT4 and NANOG expression showed a significant positive association with YAP expression (P= 0.030 and P=0.035) and were also associated with a higher ki-67 (P= 0.010) and with lymphovascular invasion (P= 0.005). On the other hand, the mRNA and protein expression of YAP did not show any statistically significant difference between the two cell lines (MCF-7, MDA-MB-231). Similarly, YAP expression did not show positive association with tumor grade, stage, Ki-67 expression, perinodal fat or lympho-vascular invasion nor with patient outcome as shown by the response to treatment after 5 years of follow up. An MTT viability assay showed that Verteporfin at 2 different concentrations sensitized MCF7 cells to tamoxifen at low doses.

Conclusions: The significant association found between YAP and stem cell markers points to a possible oncogenic role of YAP in breast cancer. This role is particularly important in luminal tumors where YAP showed a significantly higher expression than TNBC tumors. However, this link between YAP and stemness needs further elucidation of the underlying mechanistic pathways in order to identify possible new therapeutic targets.

177 Visualization of Benign Breast Lesions by Nonlinear Microscopy

Yaileen Guzman-Arocho[1], Tadayuki Yoshitake[2], Lucas Cahill[3], Tejas Mehta[1], Mary Jane Houlihan[1], Liza Quintana[1], James G. Fujimoto[2], James Connolly[4]
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Disclosures: Yaileen Guzman-Arocho: None; Tadayuki Yoshitake: None; Lucas Cahill: None; Tejas Mehta: None; Mary Jane Houlihan: None; Liza Quintana: None; James G. Fujimoto: None; James Connolly: None

Background: Nonlinear microscopy (NLM) is an emerging imaging technique that generates images similar to hematoxylin and eosin (H&E) from freshly excised tissue. NLM has been used to examine breast carcinoma (Cahill et al. 2018). However, the ability of NLM to characterize benign breast lesions has not been fully demonstrated.

Design: Sections of freshly excised, unsectioned breast tissue were stained with fluorescent nuclear (acridine orange) and stromal/cytoplasmic (sulforhodamine) dyes for 2 minutes, rinsed with saline for ~30 seconds, placed onto a specimen holder with a glass window, and then evaluated in real-time using a custom-designed NLM instrument at magnifications ranging from 5X to 20X, analogous to standard light microscope. The real-time evaluation was recorded and subsequently compared with standard paraffin-embedded H&E histology.

Results: We evaluated 81 breast specimens to characterize benign breast tissue types. Normal ducts and lobules with two distinctive cell types were identified in all cases. Distinctive eosinophilic cytoplasm, round nuclei, and prominent nucleoli, consistent with apocrine metaplasia were visualized. An intraductal proliferation of haphazardly oriented cells was seen in usual ductal hyperplasia. Multinucleated giant cells were identified at biopsy sites. Well-circumscribed fibroepithelial lesions including fibroadenoma and phyllodes tumor were distinguished. Cystic spaces lined by single or multiple columnar cells with elongated nuclei were identified corresponding to columnar cell changes/columnar cell hyperplasia. Papillary fronds projections were seen in intraductal papillomas. Entrapped glands with usual ductal hyperplasia moving away from a central nidus were seen in the radial scar. Slit-like spaces lined by stromal myofibroblasts consistent with pseudoangiomatous stromal hyperplasia were identified. The cytological and architectural features of benign breast lesions visualized by NLM were confirmed when compared to the respective H&E slides. Examples of NLM images and corresponding H&E slides are shown in the Figure 1.

Figure 1 - 177

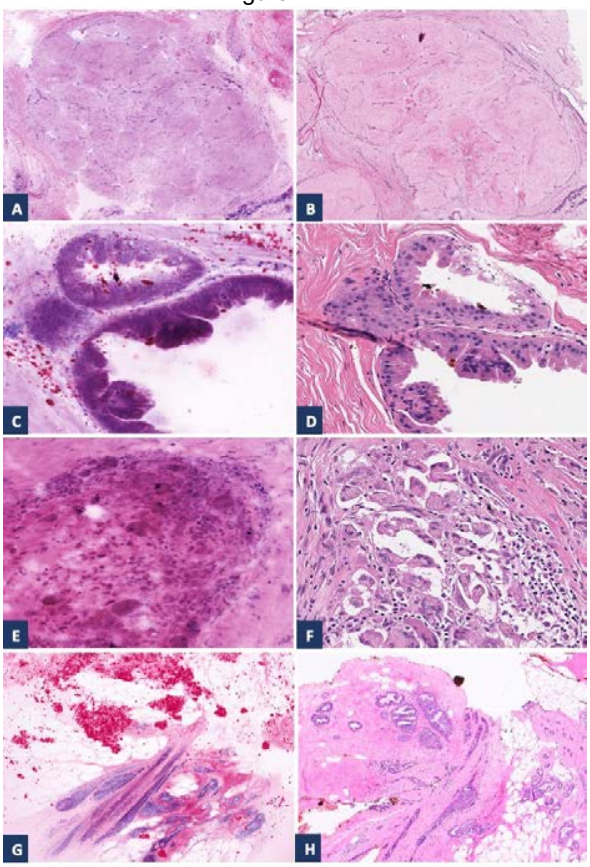

Figure: Examples of NLM images (left) and corresponding H&E slides (right) of benign breast lesions. Fibroadenoma (A and B), apocrine metaplasia (C and D), biopsy site changes (E and F), and radial scar (G and H).

Conclusions: NLM facilitates the rapid histological evaluation of freshly excised tissue and visualization of histological features necessary to characterize benign breast lesions without introducing processing artifacts.

178 Nonlinear Microscopy: Evaluation of Invasive Breast Carcinoma

Yaileen Guzman-Arocho[1], Tadayuki Yoshitake[2], Lucas Cahill[3], Tejas Mehta[1], Mary Jane Houlihan[1], Liza Quintana[1], James G. Fujimoto[2], James Connolly[4]

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Disclosures: Yaileen Guzman-Arocho: None; Tadayuki Yoshitake: None; Lucas Cahill: None; Tejas Mehta: None; Mary Jane Houlihan: None; Mary Jane Houlihan: None; Liza Quintana: None; James G. Fujimoto: None; James Connolly: None

Background: Nonlinear microscopy (NLM) is a laser-based imaging technique that allows real time evaluation of freshly excised tissue. NLM produces images similar to formalin fixed, paraffin embedded H&E histology, but without the need for freezing, fixation, embedding or slide preparation. In a prior study, we demonstrated high sensitivity (95.4%) and specificity (93.3%) for detecting breast carcinoma from benign breast tissue (Tao et al. 2014). However, the ability of NLM to further characterize invasive carcinoma subtypes has not been adequately examined. Here we aim to demonstrate the ability of NLM to assess histologic details of invasive breast cancer subtypes.

Design: Freshly excised breast tissue was serially sliced, and representative slices of tumors were selected for evaluation. The tissue was stained with fluorescent nuclear (acridine orange) and stromal/cytoplasmic (sulforhodamine) dyes for 2 minutes, followed by a ~30-second saline rinse. Tissue slices were evaluated in real-time using a custom-designed NLM instrument at magnifications ranging from 5X to 20X. NLM generates images by scanning a femtosecond laser that excites the nuclear/cytoplasmic fluorescent dyes only at the laser focus. This produces an optical sectioning effect that visualizes a thin tissue layer within a thick specimen without requiring physical sectioning with a microtome. The NLM images were displayed in an H&E-like colormap using fluorescent signals from the nuclear and cytoplasmic channels, minimizing the training required to evaluate them. The real-time evaluation was recorded and subsequently compared with standard H&E.

Results: We evaluated 20 partial mastectomy specimens with known invasive carcinoma on biopsy to further characterize the histologic subtypes of invasive carcinoma. Histological features of invasive ductal carcinoma, ranging from well-formed glands to solid sheets of cells without glands formation were identified. Single cells invading the stroma were visualized on invasive lobular carcinoma. Clusters of cells within pools of extracellular mucin was identified on mucinous carcinoma. Cytological features including nuclear pleomorphism and nuclear atypia were identified. The ability of NLM to visualize cytological and architectural features of invasive carcinoma of the breast was confirmed when compared to respective H&E slides (Figure 1).

Figure 1 - 178

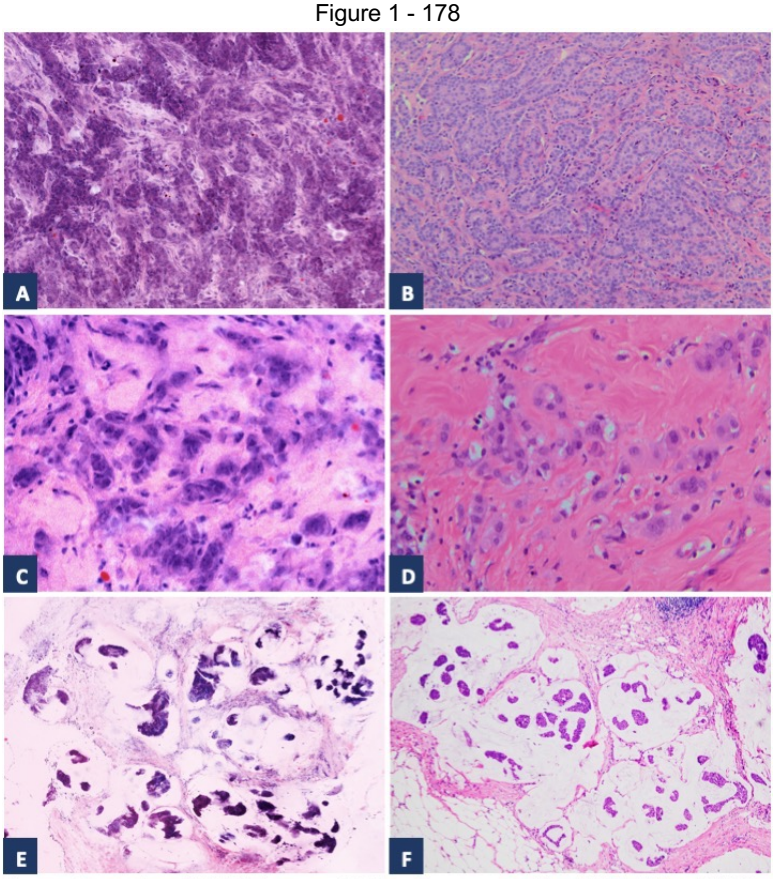

Figure: Examples of NLM images (left) and corresponding H&E slides (right). Invasive ductal carcinoma (A and B), invasive lobular carcinoma (C and D), and mucinous carcinoma (E and F).

Conclusions: NLM enables rapid assessment of breast tissue for the presence of invasive carcinoma and identification of histologic subtypes.

179 Breast Histopathologic Features Associated with Long-Term Testosterone Therapy in Transgender Individuals

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Disclosures: Yaileen Guzman-Arocho: None; Vanessa Bret-Mounet: None; Michael Pyle: None; Vanda Torous: None; Stuart Schnitt: None; Laura Collins: None; Adam Tobias: None; Valerie Fein-Zachary: None; Gerburg Wulf: None; Yujing Jan Heng: None; Gabrielle Baker: None

Background: The number of transgender (TG) individuals pursuing testosterone therapy (TT) to enhance masculinization is increasing. Although TT is generally considered safe, the impact of long-term, high-dose testosterone (T) on breast biology remains unclear. While previous studies provided insight into the complex interplay of exogenous T and endogenous female hormones in the breast, they were descriptive and did not evaluate the association of histologic features with duration of TT. This study aims to identify histologic features associated with long-term TT.

Design: We reviewed H&E slides from 318 TG individuals who underwent chest-contouring surgery at our institution (January 2013-December 2018) for various histologic findings including degree of lobular atrophy as well as atypical and non-atypical proliferations. Patient data were retrieved from medical records. Information about the duration of TT was available for 298 cases. Thus, the association between histologic features and length of TT was only assessed in 298 cases using non-parametric tests.

Results: The mean duration of TT was 22.8 months among 249 (84%) TG individuals taking T; 49 (16%) did not use T prior to surgery. Longer TT was associated with increasing degrees of lobular atrophy— median durations of TT were 10.0 months for the minimal category, 14.5 for mild, 15.3 for moderate, and 24.8 for marked (p<0.001). Duration of TT in cases without fibroadenomatous change, cysts, secretory change, or pseudoangiomatous stromal hyperplasia were significantly longer by 6.5%, 17.2%, 87.9%, and 48.5% months, respectively, compared to cases with these features (p<0.05). Five out of 318 cases (1.6%) had atypical hyperplasia (atypical ductal hyperplasia [ADH] in 2, atypical lobular hyperplasia [ALH] in 2, and both ADH and ALH in 1). The duration of TT in 3 of these individuals was 12.1, 25.6, and 64.1 months (TT duration was unavailable for the other 2); 2/5 have a family history of breast cancer and none of these patients had genetic testing conducted. Ductal carcinoma *in situ* (DCIS) was identified in 1 individual who was on TT for 61.4 months. Although this patient has a family history of male breast cancers, no germline mutation was identified upon genetic testing.

Conclusions: TT modulates hormone-related breast histopathologic features. The finding of DCIS warrants the establishment of culturally sensitive breast cancer screening protocols for TG individuals, especially for those not undergoing chest-contouring surgery.

180 SOX10 Expression in Primary and Brain Metastatic Breast Carcinoma: Evaluation of its Prognostic Value and Clinical Outcome

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Disclosures: Aparna Harbhajanka: None; Hamza Gokozan: None; Hannah Gilmore: None

Background: Brain metastasis from breast carcinoma is increasing. There is need to evaluate tumor prognostic markers, histological subtypes, and clinical outcome to better understand this dismal complication. Recently, SOX10 expression has been reported positive in both benign breast myoepithelial cells and primary breast carcinomas (PBC), especially basal-like, triple-negative breast carcinoma (TNBC). However, there is limited literature on the utility of SOX10 expression in brain metastatic breast carcinomas (BMBC).

Design: In this study, we evaluated SOX10 expression in surgically resected BMBC and PBC from 121 specimens of 76 patients sampled on tissue microarrays. There were a total 99 specimens from brain metastases including 83 BMBC and 16 from other sites.

Results: SOX10 expression was seen in 10/99 (10.1%) cases of all metastatic breast carcinomas, all were grade II/III, TNBC, metastatic to the brain (n=7) or skin (n=3) (Table 1). Overall, 10/39 (25.6%) of TNBC metastases were SOX10+, compared to 0% of estrogen receptor (ER)+ or human epidermal growth factor 2 (HER-2)+ metastases (P<.001). In patients with matched PBC and BMBC tissue (n=22), SOX10 expression was present in 13.6% (3/22) of all evaluated PBC and seen only in TNBC subgroup (fig 1). The time to BMBC was shorter with SOX10+ tumors (Log rank, P<0.014). Overall survival after BM was shorter in patients with Sox10+ tumors(fig 2).

Table 1-3. Clinicopathological characteristics of Brain metastatic breast carcinoma

Clinicopathological parameters	SOX10 Negative	SOX10 positive	Total	P value
Age((yr, mean ± SD)	54.8±11.6	54.33±12.89	-	0.693
IHC based subtypes				
ER positive	25 (100%)	0	25(33.8%)	0.001
Her2 Positive	25 (100%)	0	25(33.8%)	
TNBC	18 (75%)	6(25%)	24(32.4%)	
Total	68	6	74	
T stage				
T1	11 (78.6%)	3(21.4%)	14 (40%)	0.626
T2	12(87.5%)	2(14.3%)	14 (40.%)	
T3	6 (100%)	0	6(17.1%)	
T4	1(3.3%)	0	1(2.9%)	
Total	30	5	35	
N stage				
N0	11(84.6%)	2(15.4%)	13(40%)	.834
N1	8(88.9%)	1(11.1%)	9(28.1%)	
N2	6(85.7%)	1(14.3%)	7(21.9%)	
N3	2(66.7%)	1(33.3%)	3(9.4%)	
Total	27	5	32	
AR				
absent	31(83.8%)	6(16.2%)	37(50.7%)	0.025
present	36(100%)	0	36(49.3%)	
Total	67	6	73	
ER				
absent	36(85.7%)	6(14.3%)	42(56.8%)	0.03
present	32 (100%)	0	32(43.2%)	
Total	68	6	74	
PR				
absent	42(87.5%)	6(12.5%)	48(64.9%)	0.085
present	26 (100%)	0	26(35.1%)	
Total	68	6	74	
HER2				
absent	42(87.5%)	6(12.5%)	48(64.9%)	0.085
present	26 (100%)	0	26(35.1%)	
Total	68	6	74	
P16				
<10%	50(100%)	0	50(67.6%)	0.001
>10%	18 (75%)	6(25%)	24(32.4%)	
Total	68	6	74	

Figure 1 - 180

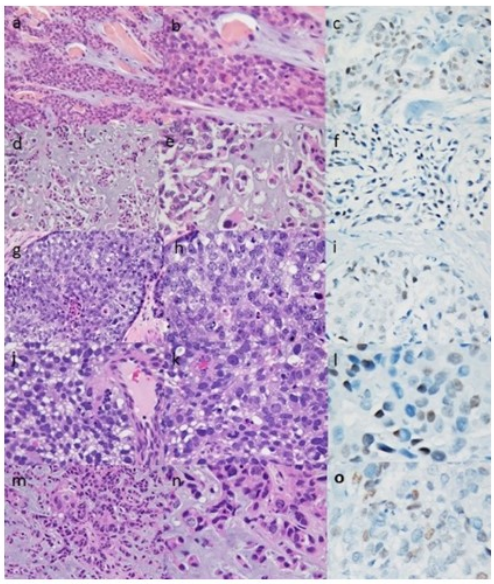

Figure 1: Case 1, a-f: a-c, Breast mass, the tumor cells show focal clear cell change and chondromyxoid background (H&E, a 200x; b, 400x); SOX10 positive triple negative breast cancer (c, SOX10 IHC, 200x,). d-e, Brain metastasis, the tumor cells show epithelioid cells with eosinophilic cytoplasm and chondromyxoid background (H&E, d 200x; e, 400x); SOX10 positive (f, SOX10 IHC, 200x,). Case 2, g-l. g-i, breast mass. The tumor cells are poorly differentiated with clear cell change and are arranged in solid nests. (H&E, g, 200x; h, 400x); SOX10 positive (i, SOX10 IHC, 400x); j-l, Brain metastasis, the tumor cells show clear cell change. (H&E, j 200x; k, 400x); SOX10 positive (l, SOX10 IHC, 400x,). Case 3, m-o, Brain metastasis, the tumor cells show epithelioid cells with eosinophilic cytoplasm and chondromyxoid background (H&E, m 200x; e, 400n); SOX10 positive (o, SOX10 IHC, 400x).

Figure 2 - 180

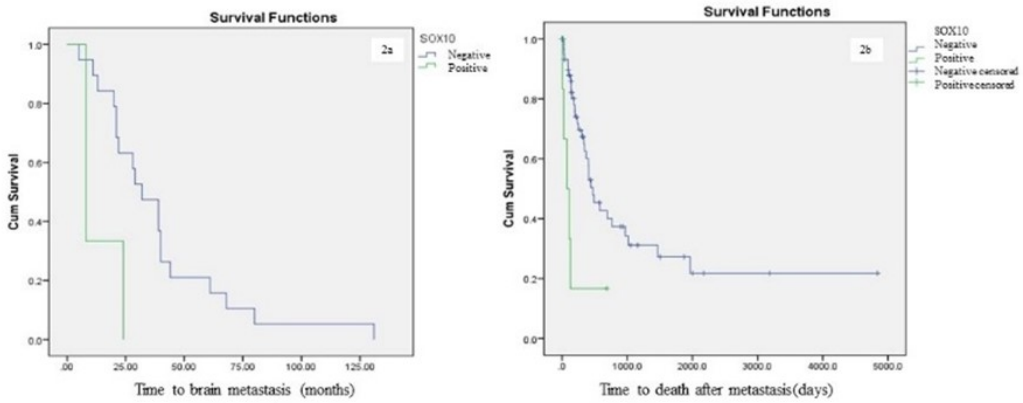

Figure 2: Time to brain metastasis (a) and overall survival (b) after metastasis in SOX10 positive and negative cases. Figure 2: Time to brain metastasis (a) and overall survival (b) after metastasis in SOX10 positive and negative cases.

Conclusions: In conclusion, SOX10 expression is seen in a subset of brain metastatic TNBC, supporting its use as a marker of breast origin and is not just limited to metastatic melanoma. The time to brain metastasis and overall survival after metastasis is shorter with SOX10+ tumors.

181 Genomic Analysis of Inflammatory Breast Cancer: A Single Institution Experience with a Large, Well-Characterized Cohort

Beth Harrison[1], Liam Spurr[2], Yvonne Li[2], Marie Claire Remolano[2], Melissa Hughes[2], Ana Garrido-Castro[2], Meredith Regan[2], Deborah Dillon[3], Allison Cleary[1], Jennifer Rosenbluth[2], Andrew Cherniack[4], Nancy Lin[2], Beth Overmoyer[2]
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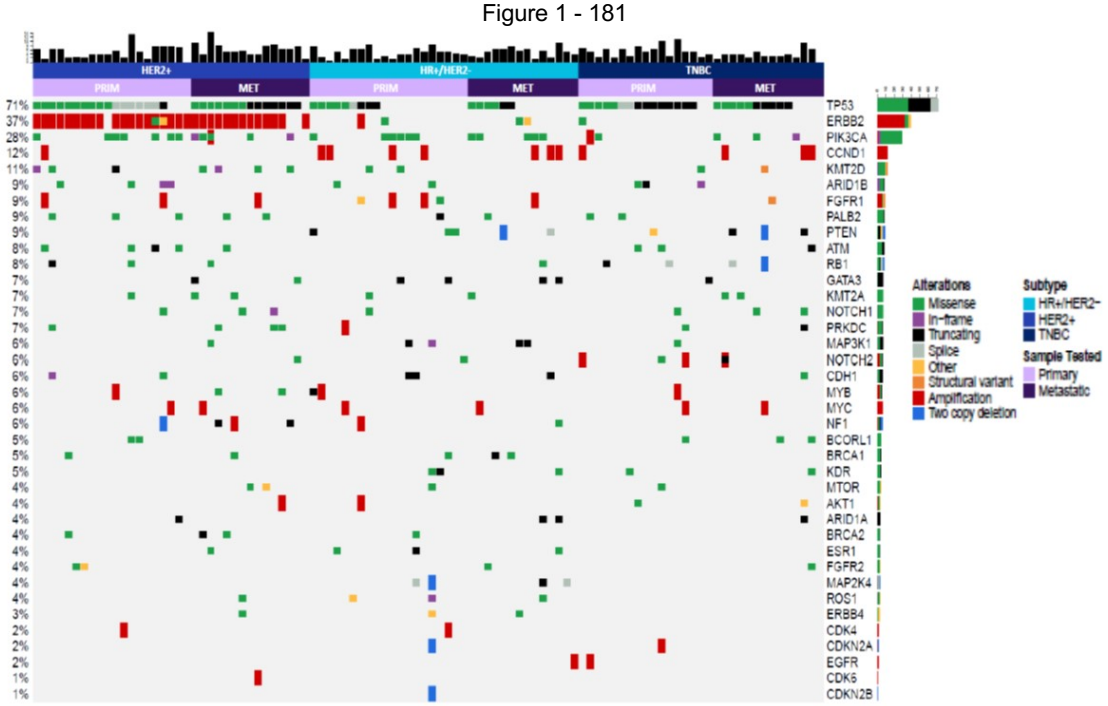
Disclosures: Beth Harrison: None; Liam Spurr: None; Yvonne Li: None; Marie Claire Remolano: None; Melissa Hughes: None; Ana Garrido-Castro: None; Meredith Regan: None; Deborah Dillon: *Advisory Board Member*, Oncology Analytics, Inc; *Consultant*, Novartis; Allison Cleary: None; Jennifer Rosenbluth: None; Andrew Cherniack: None; Nancy Lin: *Grant or Research Support*, Genentech; *Grant or Research Support*, Merck; *Grant or Research Support*, Seattle Genetics; *Grant or Research Support*, Pfizer; *Consultant*, Daichii Sankyo; Beth Overmoyer: None

Background: Inflammatory breast cancer (IBC) is an aggressive form of breast cancer (BC) characterized by breast erythema and edema secondary to tumor emboli within skin lymphatics. Due to the rarity of IBC and challenges in its diagnosis, much remains to be determined regarding its molecular pathogenesis. We investigated the genomic profile of tumors from IBC patients (pts) seen at the IBC Program at DFCI.

Design: Pts who consented to NGS-based tumor profiling (OncoPanel,OP) from 2013-2019 were identified from an IRB approved IBC registry. OP v1-v3.1 interrogated full coding sequences of 275-447 genes for mutations (muts) and copy number variations. Alterations common to all OP versions were compared between subgroups using Fisher's exact test ($p<0.05$) with FDR adjustment ($q<0.25$). Cox proportional-hazards models controlling for BC subtype were employed for overall survival (OS) analysis.

Results: 101 IBC pts had OP performed on a single tumor sample. Samples tested were primary (57) or metastatic (44) and HR+/HER2- (34), HER2+ (35) or triple negative (TN) (31). IBC tumors harbored several recurrent muts (see Figure 1) including likely oncogenic muts in *TP53* (53%), *PIK3CA* (20%), *GATA3* (6%), *PTEN* (5%), *ARID1A* (4%), *RB1* (4%), *CDH1* (3%), *MAP2K4* (3%), *MAP3K1* (3%), *ATM* (2%), *CREBBP* (2%), *ESR1* (2%) and *NF1* (2%). Recurrent gene amplifications (amps) involved *ERBB2* (33%), *CCND1* (12%), *FGFR1* (6%), *MYC* (6%), *RARA* (5%), *RAD21* (4%) and *NOTCH2* (3%). HER2+ and TN tumors were enriched for *TP53* muts compared to HR+/HER2- (71.4% and 61.3% vs 29.4%;p=0.001,q=0.067). Tumors from pts who had secondary IBC in an ipsilateral breast previously treated for BC were enriched for *PIK3CA* muts (57% vs 22%;p=0.01) and those from pts who were stage IV at presentation were enriched for *ERBB2* amp (48% vs 19%;p=0.003), although findings lost significance after FDR correction. No other significant correlations were found between mutation burden or alterations and clinicopathologic features such as age, menopausal status, sample type tested, tumor histology, LVI or site of metastasis. *ERBB2* amp was the only alteration associated with longer OS, while *FBXW7* mut was associated with shorter OS.

Figure 1 - 181



Conclusions: IBC features a diverse repertoire of genomic alterations that overlaps with those previously implicated in BC with a high frequency of *TP53* muts suggesting a more aggressive biologic phenotype. Future analysis will compare IBC and non-IBC tumors profiled at our institution to further investigate a potential IBC-specific phenotype.

182 Can IHC4 Profile Help Predict a Low Oncotype Recurrence Score (RS) in Clinically Low Risk Breast Tumors?

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Disclosures: Daniel Harter: None; Pavankumar Tandra: None; Jairam Krishnamurthy: None; Yun-An Tseng: None

Background: Oncotype DX is a 21 gene RT-PCR based assay commonly used for prognosis and predicting the benefit of chemotherapy treatments in ER+, HER2- breast cancer with either node negative or 1-3 node-positive (LN+ 1-3) disease. The purpose of this study focuses on investigating the trend of IHC4 (ER, PR, HER2 and Ki-67) profile in Oncotype low risk (RS 1-10) and intermediate risk (RS 11-25) groups and if the IHC4 profile can help predict an Oncotype RS of 10 or less.

Design: 654 newly diagnosed breast cancers were retrieved from the 2018 tumor archives. 54 of them were sent for Oncotype and 51 cases had sufficient tumor for analysis. The 51 cases were divided into clinical low (c-low: grade 1 ≤ 3 cm, grade 2 ≤ 2 cm, grade 3 ≤ 1 cm, grade 1 ≤ 2 cm with LN+ 1-3) and clinical high risk (c-high: grade 1 > 3 cm, grade 2 > 2 cm, grade 3 > 1 cm, grade 1 > 2 cm with LN+ 1-3 and grade 2-3 with LN+ 1-3) groups. The results of immunohistochemical testing of ER, PR, HER2 and Ki-67 and Oncotype recurrence score were recorded for each case.

Results: Among 51 cases, 25 cases had low risk RS (1-10), 23 cases had intermediate RS (11-25) and 3 cases had high risk RS (26-100). 19 (76%) low risk RS cases are c-low with 18 (95%) cases showing ER ≥ 90%, HER2- and Ki-67 ≤ 14%. 6 (24%) low risk RS cases are c-high with 4 (67%) cases showing ER ≥ 90%, HER2- and Ki-67 ≤ 14%. 18 (78%) intermediate risk cases are c-low with 11 (61%) cases showing ER ≥ 90%, HER2- and Ki-67 ≤ 14%. 5 (22%) intermediate risk cases are c-high with 3 (60%) cases showing ER ≥ 90%, HER2- and Ki-67 ≤ 14%. None of the high risk RS cases are c-low in this study or show a combination IHC profile of ER ≥ 90%, HER2- and Ki-67 ≤ 14%.

Oncotype RS score	Low (0-10)	Intermediate (11-25)	High (26-100)
Case number (n=51,%)	25 (49)	23 (45)	3 (6)
Histologic (Nottingham) grade			
Low (1) (n,%)	11 (44)	6 (26)	0 (0)
Intermediate (2) (n,%)	12 (48)	16 (69)	0 (0)
High (3) (n,%)	2 (8)	1 (4)	3 (100)
Tumor size			
≤ 1 cm (n,%)	6 (24)	3 (13)	0 (0)
1.1 cm - 2cm (n,%)	15 (60)	16 (70)	0 (0)
2.1 cm - 3 cm (n,%)	2 (8)	3 (13)	3 (100)
> 3 cm (n,%)	2 (8)	1 (4)	0 (0)
Lymph node status			
LN 0 (n,%)	23 (92)	21 (91)	2 (67)
LN+ 1-3 (n,%)	2 (8)	2 (9)	1 (33)
LN+ > 3 (n,%)	0 (0)	0 (0)	0 (0)
Clinical risk - low (n,%)	19 (76)	18 (78)	0 (0)
Estrogen receptor (ER) status (%)			
≥ 90 (n,%)	19 (100)	17 (94)	0 (0)
76-89 (n,%)	0 (0)	1 (6)	0 (0)
51-75 (n,%)	0 (0)	0 (0)	0 (0)

26-50 (n,%)	0 (0)	0 (0)	0 (0)
1-25 (n,%)	0 (0)	0 (0)	0 (0)
Progesterone receptor (PR) status (%)			
≥ 90 (n,%)	13 (68)	10 (56)	0 (0)
76-89 (n,%)	2 (10)	0 (0)	0 (0)
51-76 (n,%)	2 (10)	3 (16)	0 (0)
26-50 (n,%)	1 (5)	0 (0)	0 (0)
1-25 (n,%)	1 (5)	5 (28)	0 (0)
HER2 by IHC			
Negative (n,%)	19 (100)	18 (100)	0 (0)
Positive (n,%)	0 (0)	0 (0)	0 (0)
Ki-67 (%)			
≤ 14 (n,%)	18 (95)	11 (61)	0 (0)
> 14 (n,%)	1 (5)	7 (39)	0 (0)
Clinical risk - high	6 (24)	5 (22)	3 (100)
Estrogen receptor (ER) status (%)			
≥ 90 (n,%)	6 (100)	5 (100)	1 (33)
76-89 (n,%)	0 (0)	0 (0)	0 (0)
51-75 (n,%)	0 (0)	0 (0)	2 (67)
26-50 (n,%)	0 (0)	0 (0)	0 (0)
1-25 (n,%)	0 (0)	0 (0)	0 (0)
Progesterone receptor (PR) status (%)			
≥ 90 (n,%)	3 (50)	2 (40)	0 (0)
76-89 (n,%)	2 (33)	0 (0)	0 (0)
51-75 (n,%)	0 (0)	1 (20)	0 (0)
26-50 (n,%)	0 (0)	2 (40)	1 (33)
1-25 (n,%)	1 (16)	0 (0)	2 (66)
HER2 by IHC			
Negative (n,%)	6 (100)	5 (100)	3 (100)
Positive (n,%)	0 (0)	0 (0)	0 (0)
Ki-67 (%)			
≤ 14 (n,%)	4 (67)	3 (60)	0 (0)
> 14 (n,%)	2 (33)	2 (40)	3 (100)

Conclusions: 96% of breast carcinomas with Oncotype RS ≤10 in this study have either low or intermediate histologic grade, size ≤ 2 cm with IHC profile of ER ≥ 90%, HER2- and Ki-67 ≤ 14%. While the same IHC profile is also observed in 61% of c-low cases in Oncotype RS11-25 group, the TAILORx trial published in August, 2018 concluded that no statistically significant benefit was found in patients with intermediate RS treated with chemotherapy although some benefit of chemotherapy was found in women 50 years or younger.

Based on our small study and the TAILORx conclusion, females above age 50 with grade 1-2, T1 N0 breast carcinomas with ER ≥ 90%, HER2- and Ki-67 ≤ 14% may possibly avoid costly molecular studies as Oncotype RS results may not change the overall management.

183 Breast Cancer with Equivocal HER2 IHC: Does HER2 IHC Intensity Identify Areas with FISH Amplification?

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Disclosures: Johann D. Hertel: None; Kathleen Kaiser-Rogers: None; Siobhan O'Connor: None; Benjamin Calhoun: *Advisory Board Member*, Luminex Corporation

Background: The 2018 ASCO/CAP HER2 guidelines eliminate the equivocal category for in situ hybridization (ISH) and emphasize concurrent interpretation of immunohistochemistry (IHC) with ISH. Evaluation of HER2 IHC using sections from the same sample used for ISH is recommended to guide the selection of areas for ISH scoring. The utility of IHC intensity for selecting areas for ISH scoring was evaluated in a series of IHC equivocal (2+) breast cancers.

Design: A total of 86 cases with equivocal HER2 IHC (2+) that were reflexed to FISH were prospectively collected from 8/22/2018 to 6/26/2019. Tumor grade, histology, hormone receptor status and variability in HER2 IHC staining intensity were recorded. The areas of most and least intense HER2 IHC staining were separately identified and scored by FISH. The mean HER2:CEP17 ratio and mean HER2 signals:tumor cell were calculated for both populations and statistical significance was evaluated using the Wilcoxon Ranked Sum test.

Results: The distribution of the high and low intensity groups is reported in Table 1. Overall, 83 of 86 (95.5%) cases sorted into the same ISH group, 7 (9.3%) sorted into different ISH groups and 3 (3.5%) had different final ISH categorizations in the high and low intensity areas. These 3 cases were ISH Group 1 (amplified) in the high intensity area and ISH group 2, 4 or 5 (not amplified in the absence of 3+ IHC) in the low intensity area. The other 4 cases with different ISH groups did not have different final HER2 categorizations. While most cases (88%) showed some degree of variability in HER2 stain intensity, all 3 discordant cases showed significant variability in the HER2 staining intensity. The mean HER2:CEP17 ratios for the high and low intensity areas were 1.48 and 1.37 (p=0.017) with no statistically significant difference in HER2 copy number (p=0.65).

Low versus high intensity ISH group distribution		
	Low Intensity	High Intensity
ISH group 1	6	9
ISH group 2	2	1
ISH group 3	0	0
ISH group 4	10	6
ISH group 5	68	70

Conclusions: When areas with high and low HER2 IHC intensity were compared, there was a small difference in the mean HER2:CEP17 ratio and a minority of cases (3.5%) showed discordant final HER2 categorization. However, the discordant cases account for 33% of the amplified cases. Cases with discordant HER2 categorization showed readily identifiable high and low intensity areas and were amplified in the IHC high intensity area and not amplified in the low intensity area. The data suggests that using HER2 IHC to guide selection of areas for ISH testing may identify amplified tumors (or clones) in a minority of cases.

184 Interobserver Variation of PD-L1 (SP142) Immunohistochemistry Interpretation in Breast Carcinoma

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Disclosures: Raza Hoda: None; Edi Brogi: None; Timothy D'Alfonso: None; Anne Grabenstetter: None; Dilip Giri: None; Matthew Hanna: None; M Gabriela Kuba: None; Melissa Murray: None; Christina Vallejo: None; Hong (Amy) Zhang: None; Jorge Reis-Filho: *Consultant*, Goldman Sachs; *Consultant*, REPARE Therapeutics; *Advisory Board Member*, Volition Rx; *Advisory Board Member*, Paige.AI; *Advisory Board Member*, Roche Tissue Diagnostics; Hannah Wen: None

Background: The PD-L1 (SP142) immunohistochemical assay (Ventana, Tucson, AZ) has been recently approved by the FDA as a companion diagnostic assay to determine eligibility of patients with locally advanced or metastatic triple-negative breast cancer (TNBC) for immunotherapy with atezolizumab, a monoclonal antibody targeting PD-L1. Thus, treatment selection rests on reliable assay interpretation; however, studies on interobserver variability among pathologists with the PD-L1 (SP142) assay remain limited. The objective of this study is to determine the interobserver variability in PD-L1 (SP142) immunohistochemistry (IHC) interpretation in breast carcinoma.

Design: The institutional database of a single large, academic cancer center was interrogated for all patients diagnosed with primary or metastatic breast cancer cases on which PD-L1 SP142 IHC was performed from 2018 to 2019. 79 of 127 retrieved cases (62%) were randomly selected for evaluation. Assessment of PD-L1 IHC in stromal tumor-infiltrating lymphocytes (TILs) was evaluated on whole slide images by 8 staff pathologists with breast pathology expertise (Figure 1). PD-L1 was scored according to the criteria in the Impassion 130

trial, as negative, where staining of TILs occupies <1% of the tumor area, or positive, where staining of TILs occupies 1% or greater of the tumor area. Interobserver variability was calculated using unweighted kappa, whereby a value of 0.01-0.20 indicated slight agreement, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 substantial agreement, and 0.81-0.99 near-perfect agreement.

Results: PD-L1 IHC was performed on 79 breast cancer cases. Clinical and pathologic features of the study cohort are summarized in Table 1. The kappa score for interobserver agreement was 0.727. There was complete agreement among all 8 pathologists in 49 cases (62%), among at least 7 pathologists in 66 cases (84%) and among at least 6 pathologists in 73 cases (92%). In 3 cases (4%), pathologists' scores were divided equally, with half of the participants scoring the case as positive and negative (Figure 2; arrowheads indicate areas of PD-L1 staining). These discrepant cases included 2 core needle biopsies [one primary (Figure 2A-B), one metastasis to liver (Figure 2C-D)] and 1 excision [metastasis to cecum (Figure 2E-F)].

Table 1. Clinical and pathologic features of 79 cases of primary and metastatic breast carcinoma from 75 patients with PD-L1 immunohistochemistry performed.

Characteristic	***n* (%)**
Gender	
Female	74 (99)
Male	1 (1)
Age at primary diagnosis, median, (mean), [range], years	49 (50) [23 – 87]
Site	
Breast	27 (34)
Chest wall	11 (14)
Liver	7 (9)
Lymph node	7 (9)
Lung	5 (6)
Skin	5 (6)
Soft tissue	5 (6)
Bone	4 (5)
Small and large intestine	3 (4)
Brain	2 (3)
Other	3 (4)
Tumor histology	
Invasive carcinoma of no special type	54 (68)
Metaplastic carcinoma	8 (10)
Invasive mammary carcinoma (mixed ductal and lobular features)	4 (5)
Invasive lobular carcinoma, pleomorphic or with pleomorphic features	4 (5)
Classic invasive lobular carcinoma	2 (3)
Invasive ductal carcinoma, apocrine features	2 (3)
Invasive ductal carcinoma, apocrine and micropapillary features	1 (1)
Invasive ductal carcinoma, micropapillary and mucinous features	1 (1)
Carcinoma with apocrine differentiation	1 (1)
Data not available	2 (3)

Figure 1 - 184

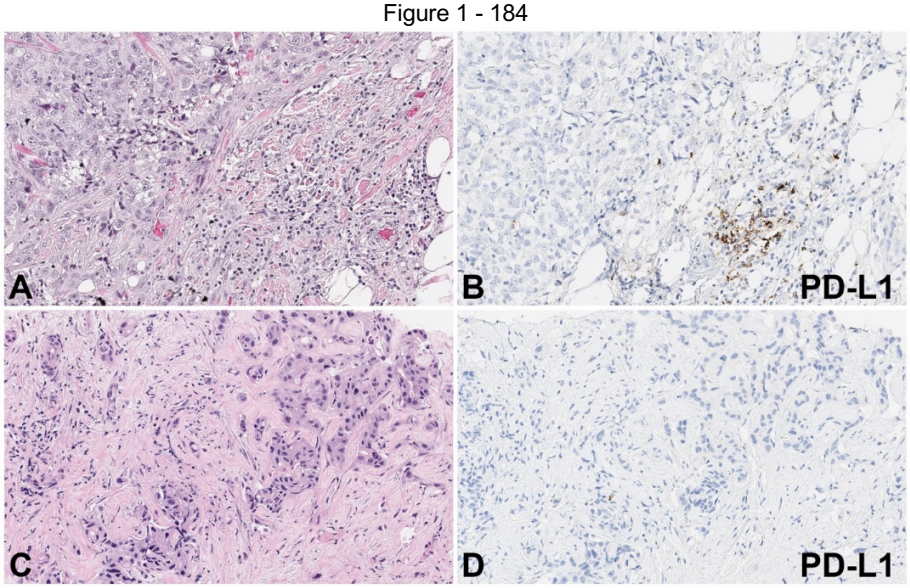
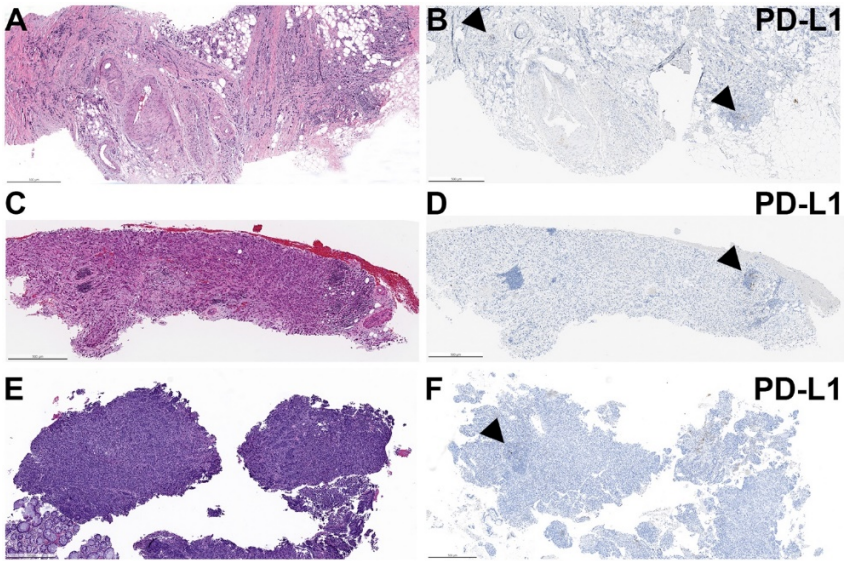


Figure 2 - 184



Conclusions: There is substantial agreement in PD-L1 (SP142) IHC assessment of breast cancer cases among 8 pathologists. Further study is warranted to define the basis of these discrepancies.

185 Next-Generation Assessment of Human Epidermal Growth Factor Receptor 2 (ERBB2) Status in Breast Cancer: A Focus on Group 4 Using the 2018 ASCO/CAP HER2 Testing Guideline

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Background: Accurate HER2 status assessment is integral to patients with breast carcinoma (BC). ASCO/CAP updated the testing guideline in 2018 to address issues from uncommon HER2 fluorescence in situ hybridization (FISH) results. This study evaluates BCs with equivocal HER2 immunohistochemistry (IHC) results (2+,1+ to 2+) with HER2 FISH and next-generation sequencing (NGS) to assess *ERBB2* amplification status, focusing on Group 4 (*HER2*/CEP17 Ratio<2.0;Average *HER2* Signals/cell >=4.0 and <6.0).

Design: BCs with equivocal HER2 IHC results from 2009-2019 were retrieved from a single institution's database. Cases were included if HER2 FISH and NGS by a hybridization capture-based assay (targeting up to 468 genes, previously validated for *ERBB2* amplification calls) were performed on the same sample. Clinical, pathologic, HER2 FISH and NGS data were reviewed. Cases were reclassified into 2018 ASCO/CAP ISH groups, and all Groups 2, 3 and 4 BCs were included. Groups 1 and 5 BCs were selected from the prior validation group. NGS results of ISH Groups 2, 3 and 4 BCs were compared to those of Groups 1 and 5. Following criteria was used to determine significance of whole-gene gain events by NGS: fold change (FC) >=2.0 (amplification), FC >=1.5 but <2 (copy number gain/borderline amplification) with *P*<.05. Copy number gains were interpreted with overall copy number profile and tumor purity.

Results: 86 BCs were reviewed, including 11 Group 1 cases (13%), 4 Group 2 (4%), 6 Group 3 (7%), 29 Group 4 (34%) and 36 Group 5 (42%). Clinical, pathologic and molecular findings are shown in Table 1. Comparison of FISH and NGS *ERBB2* amplification calls is shown in Fig.1. HER2 FISH and NGS showed complete concordance in Groups 1 and 5. Groups 2 and 3 show varied NGS results but limited by case numbers. Group 4 had 97% concordance with HER2 FISH and NGS *ERBB2* results. 1 case in Group 4 (*HER2*/CEP17 ratio:1.6; *HER2* signals/cell:5.2) showed amplification by NGS (FC:1.98, *P*=.001); FISH testing on an alternate sample of this cases revealed amplification (*HER2*/CEP17 ratio:2.4;*HER2* signals/cell:5.3). NGS showed no significant differences in genetic mutations in Groups 4 and 5 cases (Fig.2). Despite most Group 4 patients (*n*=21;72%) not receiving HER2-targeted therapy, 86% were alive at follow-up.

Table 1. Clinical, pathologic and molecular characteristics of 86 primary and metastatic breast carcinoma cases with equivocal HER2 immunochemistry results by American Society of Clinical Oncology/College of American Pathologists HER2 in situ hybridization group.

	2018 American Society of Clinical Oncology/College of American Pathologists HER2 ISH Group				
Feature	**Group 1 (*n* = 11)**	**Group 2 (*n* = 4)**	**Group 3 (*n* = 6)**	**Group 4 (*n* = 29)**	**Group 5 (*n* = 36)**
Age, median (range), years	53 (34–67)	53 (35–74)	51 (42–72)	52 (28–79)	51 (26–80)
Specimen type, No. (%)					
Primary tumor	1 (9)	0	4 (67)	19 (66)	19 (53)
Metastatic tumor	10 (91)	4 (100)	2 (33)	10 (24)	17 (47)
HER2/CEP17 ratio, median (range)	2.4 (2.1–8.4)	2.3 (2.1–4.0)	1.5 (0.8–1.7)	1.6 (1.0–1.9)	1.2 (1.0–1.7)
HER2 copy number median (range)	6.3 (4.3–18.0)	3.4 (2.1–3.9)	6.3 (6.0–7.6)	4.3 (4.0–5.9)	2.8 (1.0–3.9)
ERBB2 fold change, median (range)	1.8 (1.5–3.2)	2.1 (1.2–3.3)	1.3 (1.0–3.1)	1.1 (-1.1–2.0)	1.0 (-1.3–1.6)
Histologic grade, No. (%)					
1	0	0	0	0	0
2	5 (45)	0	2 (33)	6 (21)	18 (50)
3	4 (36)	4 (100)	4 (67)	21 (72)	10 (28)
Data not available	2 (18)	0	0	2 (7)	8 (22)
Hormone receptor status, No. (%)					
ER+, PR+	6 (55)	3 (75)	5 (83)	17 (59)	25 (69)
ER+, PR-	3 (27)	1 (25)	1 (17)	6 (21)	7 (19)
ER-, PR+	0	0	0	0	0
ER-, PR-	1 (9)	0	0	6 (21)	3 (8)
Data not available	1 (9)	0	0	0	1 (3)
Tumor histology, No. (%)					
Invasive carcinoma of NST	5 (45)	5 (100)	4 (67)	23 (79)	22 (61)
IDC with apocrine features	0	0	1 (17)	0	3 (8)
IDC with micropapillary features	0	0	1 (17)	0	0
IDC with mucinous features	2 (18)	0	0	2 (7)	1 (3)
Classic ILC	0	0	0	0	3 (8)
Pleomorphic ILC or ILC with pleomorphic features	2 (18)	0	0	0	2 (6)
Mixed ductal and lobular	1 (9)	0	0	1 (3)	0
Data not available	1 (9)	0	0	2 (7)	5 (14)
Mutations, No./Total (%)					
TP53	3/10 (30)	1/4 (25)	3/6 (50)	11/28 (39)	12/35 (34)
PIK3CA	2/10 (20)	0/4 (0)	2/6 (33)	8/24 (29)	15/35 (43)
GATA3	2/10 (20)	2/4 (50)	2/6 (33)	5/28 (18)	8/35 (23)
GLI1	0/10 (0)	0/4 (0)	2/6 (33)	1/28 (4)	0/35 (0)
ESR1	2/10 (20)	0/4 (0)	2/6 (33)	1/28 (4)	3/35 (9)
Treatment with HER2 targeted therapy, No. (%)					
Yes					
Neoadjuvant ± adjuvant	3 (27)	3 (75)	3 (50)	2 (7)[b]	1 (3)
Adjuvant	1 (9)	0	1 (17)	6 (21)[b]	2 (6)
No	6 (55)	1 (25)	2 (33)	21 (72)	32 (89)
Data not available	1 (9)	0	0	0	1 (3)
Treatment response, No. (%)					
Pathologic complete response	0	0	0	0	0
Residual invasive carcinoma	3 (100)	1 (33)	1 (33)	2 (100)	1 (100)
Primary not resected	0	2 (67)	2 (67)		0
Clinical follow-up time, median (range), months	52.0 (13.0-80.2)	35.3 (2.1-53.1)	24.2 (6.5-54.6)	22.9 (1.4-38.0)	29.8 (3.2-114.2)
Clinical status, No. (%)					
Alive	4 (36)	3 (75)	4 (67)	25 (86)	19 (53)
Died of Disease	7 (64)	1 (25)	2 (33)	4 (14)	16 (44)
Data not available	0	0	0	0	1 (3)

Abbreviations: HER2, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ISH, in situ hybridization; N/A, not applicable; NST, no special type

[a] The case with FC of 1.6 did not show significance (*P*=.09) and was not called as a gain in the clinical report.

[b] Two of 8 patients in Group 4, who received targeted HER2 therapy, showed HER2 amplification by fluorescence in situ hybridization testing on an alternate specimen.

Figure 1 - 185

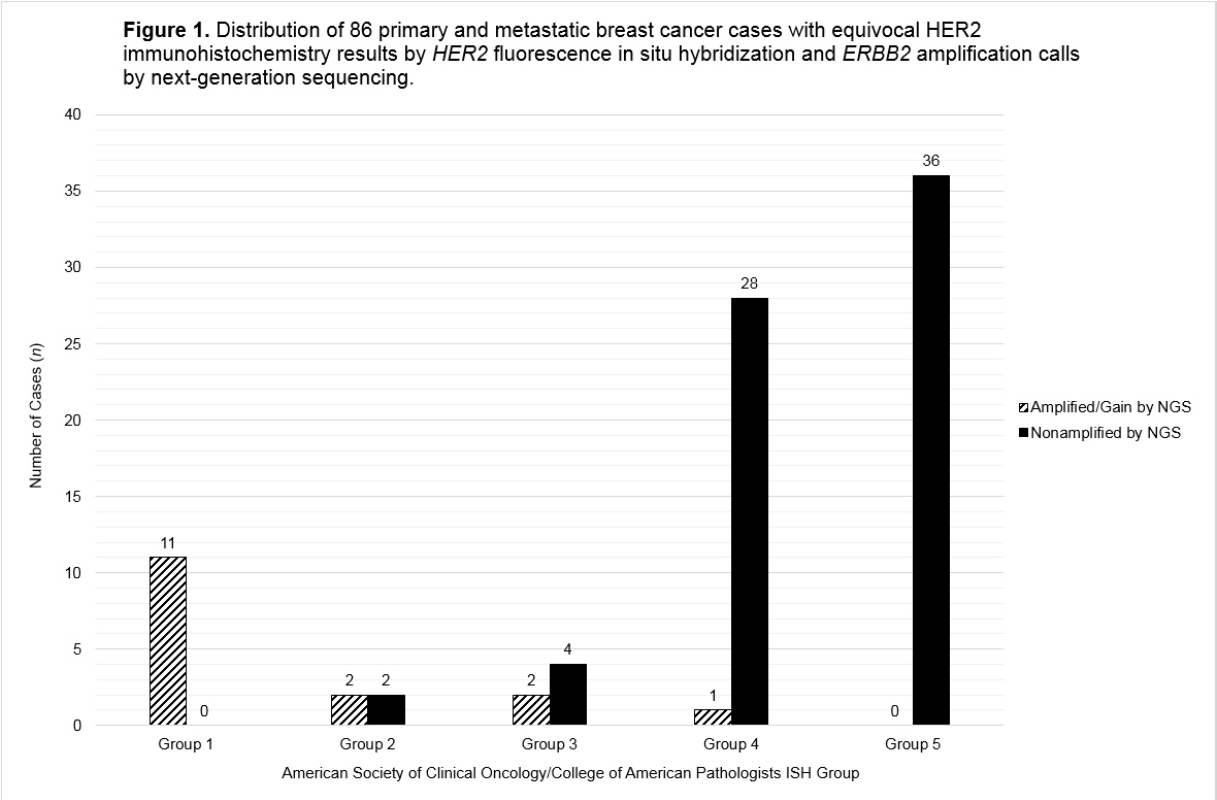
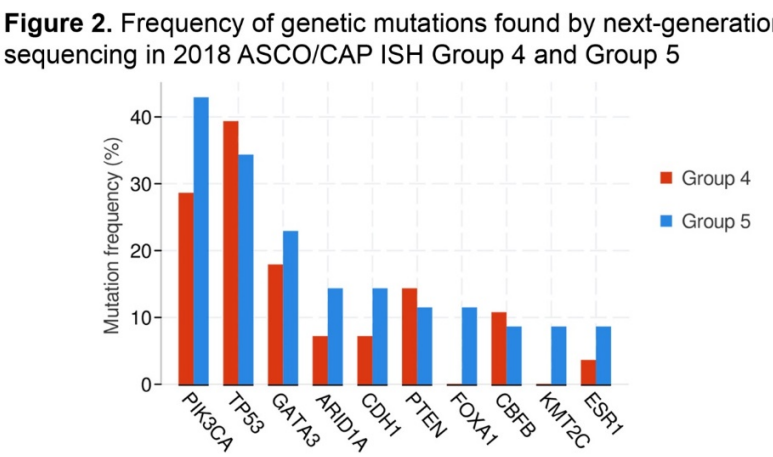


Figure 2 - 185



Conclusions: Our study demonstrates Group 4 cases are similar to Group 5 in NGS *ERBB2* amplification and mutational assessments. Our results find no support for HER2-targeted therapy in Group 4 patients, who were previously in a gray zone.

186 PD-L1 Expression in Tumor-Infiltrating Lymphocytes of Invasive Breast Carcinoma

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Disclosures: Raza Hoda: None; Edi Brogi: None; Anne Grabenstetter: None; Sujata Patil: None; Pier Selenica: None; Britta Weigelt: None; Jorge Reis-Filho: *Advisory Board Member*, Roche Tissue Diagnostics; *Advisory Board Member*, Ventana Medical Systems; Tiffany Traina: *Consultant*, Genentech/Roche; *Consultant*, Merck; *Consultant*, Celgene; Mark Robson: None; Larry Norton: None; Hannah Wen: None

Background: Following approval of atezolizumab for patients with locally advanced or metastatic triple negative breast cancer (TNBC) with PD-L1-positive immune cells, PD-L1 immunohistochemistry (IHC) assessment with SP142 assay has been widely implemented. The Ventana SP142 assay is the FDA-approved companion diagnostic for atezolizumab therapy selection. We aim to elucidate clinical, pathologic and molecular findings associated with PD-L1 expression in breast cancer (BC).

Design: We validated PD-L1 SP142 assay at our center in 11/2018 and assess PD-L1 expression in primary, recurrent or metastatic BCs as requested by treating physicians. We identified BCs with SP142 IHC performed between 11/2018-7/2019. Clinical and pathologic data and available molecular results were reviewed. Tumor infiltrating lymphocytes (TILs) were evaluated per International TILs Working Group recommendations. PD-L1 scoring followed the IMpassion 130 trial criteria, with >=1% considered positive.

Results: 128 BC samples (121 patients) were evaluated with PD-L1 SP142, including 81 TNBCs, 3 ER-/HER2+ and 34 ER+/HER2- cases. Overall 32% were PD-L1 positive. PD-L1 positivity was seen more frequently in TNBCs than non-TNBCs but was not statistically significant (39% vs 20%;*P*=.063). PD-L1 expression was significantly more frequent in primary BCs than in metastases (43% vs 25%;*P*=.04). No significant difference was observed in PD-L1 expression among metastatic sites. 2 patients had paired primary and metastatic samples; PD-L1 was negative in both pairs. Among 81 TNBCs (27 primary BCs,56 recurrences/metastases), PD-L1 was positive in 40% and not associated with androgen receptor status (*P*=.17), metastatic site (*P*=.75) or genetic alterations (Fig.2). Among 45 primary BCs of all receptor subtypes (Table 1), PD-L1 expression was associated with high TIL percentage (*P*=.002) and metaplastic histology (*P*=.03;Fig.1A-B). 3 invasive lobular carcinomas were tested and were all PD-L1 negative (Fig.1C-D). PD-L1 expression was not significantly associated with patient age, tumor size, nodal status, histologic grade and TNBC status.

Table 1. Comparison of clinical and pathological features of 45 primary breast cancers by PD-L1 expression in tumor infiltrating immune cells

Characteristic	**PD-L1 Negative Cases, *n* (%)**	**PD-L1 Positive Cases, *n* (%)**	***P***
No. of cases	25	20	
Gender			>.99
Female	24 (96.0)	20 (100)	
Male	1 (4.0)	0	
Age, mean (SD), median (range), years	49 (12); 48 (32 – 81)	49 (14); 50 (21 – 87)	.57
Tumor size, mean (SD), median (range), cm	3.00 (2.88); 2.9 (0.8 – 10.1)	3.08 (2.49); 2.55 (1.5 – 12.0)	.24
Lymph node status			.34
Positive	5 (26)	8 (47)	
Negative	14 (74)	9 (53)	
Data not available	6	3	
Tumor histology			.027*
Invasive carcinoma of NST	14 (61)	12 (63)	
Invasive lobular carcinoma	3 (13)	0	
Metaplastic carcinoma	1 (4.3)	6 (32)	
Other	5 (22)	1 (5)	
Data not available	2	1	
Histologic grade			.15
1	0	0	
2	8 (32)	2 (10)	
3	17 (68)	18 (90)	
Triple-negative ER/PR/HER2 Status			.17
Yes	12 (50)	15 (75)	
No	12 (50)	5 (25)	
Data not available	1	0	
Tumor-infiltrating lymphocytes			.002*
Mean (SD); median (range), percentage	10 (9); 10 (0 – 40)	28 (23); 15 (5 – 70)	
Data not available	0	1	
Next-generation sequencing			.73
Performed	15	13	
Not performed	10	7	
TP53 mutation			.054
Present	5 (33)	10 (77)	
Absent	10 (67)	3 (23)	
Microsatellite instability score			>.99
Stable	11 (92)	10 (91)	
Indeterminate	1 (8)	1 (9)	
Data not available	13	9	

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; NST, no special type; PD-L1, programmed death ligand 1; PR, progesterone receptor; SD, standard deviation.
* denotes a statistically significant *P* value <.05

Figure 1 - 186

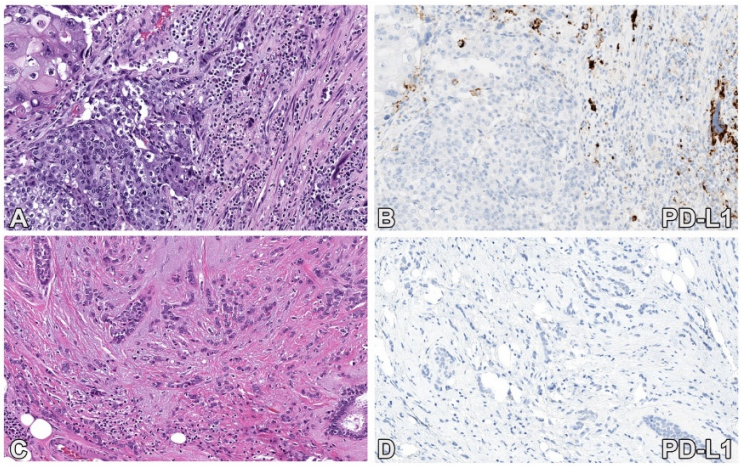
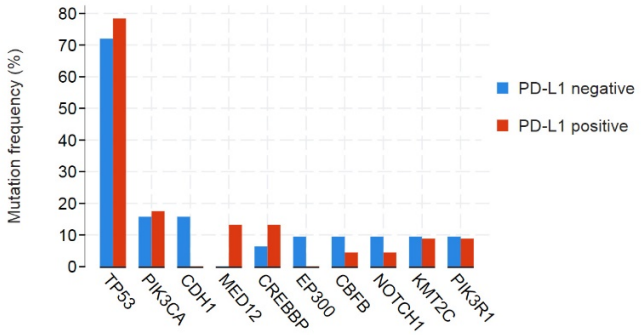


Figure 2 - 186

Figure 2. Comparison of genetic alterations found by next generation sequecing in 55 primary and metastatic triple negative breast cancer cases by PD-L1 expression with SP142 assay



Conclusions: In our study cohort, 32% of primary and metastatic BCs were PD-L1 positive using the SP142 assay. PD-L1 expression was more frequent in primary BCs than in metastatic lesions, reflecting potential differences in immune microenvironment. Histologic features associated with PD-L1 expression in primary BCs included metaplastic carcinoma and increased TILs. Further studies are needed to identify other features.

187 A Pilot Retrospective Study on the Association between Intratumoral Heterogeneity and Signal Numbers of HER2-CISH and Treatment Outcomes of Neoadjuvant Chemotherapy in HER2-Positive Invasive Breast Cancer Patients

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Disclosures: Yinan Hua: None; Arundhati Rao: None; Bing Leng: None

Background: Neoadjuvant chemotherapy is extensively adopted to treat HER2-positive invasive breast cancer, resulting in a high rate of pathologic complete response (pCR). Whereas, a portion of patients still have residual tumors after neoadjuvant therapy. The aim of the study is to investigate the factors contributing to the varied outcomes of neoadjuvant therapy, with a focus on intratumoral heterogeneity (ITH) and signal numbers of HER2.

Design: HER2 chromogenic in situ hybridization (CISH) on biopsies from twenty-five HER2-positive invasive breast cancer patients receiving neoadjuvant therapy with Taxotere, Carboplatin and Herceptin (TCH), or TCH plus Perjeta (TCHP) are reviewed in the study. ITH-HER2 is defined as one area of tumor that is HER2 negative, or HER2 positivity in >5% and <50% of tumor cells. Residual cancer burden (RCB) on the breast excision specimens are calculated.

Results: Among 25 patients, 11 patients have pCR (44%), 6 patients are classified as RCB-I (24%), 6 patients are classified as RCB-II (24%), and 2 patients RCB-III (8%). ITH-HER2 is identified in 7 patients (7/25, 28%). In ITH-HER2 group, 3 patients achieve pCR (42.9%), 2 patients have RCB-I (28.6%), 2 patients with RCB-II (28.6%) and none with RCB-III (0%). For 17 patients with high HER2 signal (>6), 58.8% (10/17) of them achieve pCR. For 8 patients with low HER2 signal (4-6), 12.5% (1/8) of them have pCR. In the pCR group, 9.1% (1/11) of patients has low HER2 signal and 90.9% (10/11) of patients have high HER2 signal ($P<0.05$). In RCB-I group, 2 patients have low HER2 signal (33.3%), and 4 have high HER2 signal (66.7%). In RCB-III group, 2 patients have low HER2 signal (100%), and none has high HER2 signal. In patients with low HER2 signal, 37.5% (3/8) of them display ITH-HER2, and for high HER2 signal patients, 23.5% (4/17) of them display ITH-HER2 ($p>0.05$). There is no significant difference in preoperative tumor size for patients with or without ITH-HER2, and for patients with low or high HER2 signal.

Conclusions: There is no significant relationship between ITH-HER2 and neoadjuvant outcomes. Interestingly, patients with high HER2-CISH signal show significant better response to neoadjuvant therapy compared to patients with low HER2-CISH signal. In addition, the ITH-HER2 ratios in low and high HER2 signal groups are not significantly different.

188 Clinicopathological Features of HER2 Positive Classic Invasive Lobular Carcinoma

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Disclosures: Xiao Huang: None; Hui Chen: None; Qingqing Ding: None; Melissa Robinson: None; Roland Bassett: None; Guilin Tang: None; Aysegul Sahin: None

Background: Compared with invasive ductal carcinoma, invasive lobular carcinoma (ILC) is larger, more common in older patients, and tends to be more commonly bilateral and multicentric. Up to 95% of ILCs express estrogen receptor (ER), 60-70% express progesterone receptor (PR) and very few express HER2.

Design: We identified women diagnosed with HER2-positive ILC between 2015 and 2019 from our pathology database. Patients' medical records were reviewed to obtain data on demographic and pathologic characteristics, including age, menopausal status, non-breast malignancy history, contralateral and concurrent ipsilateral breast lesions, disease site, clinical and pathological stage, imaging study findings, lymph node metastasis, Nottingham histological grade, histological type, HER2 immunohistochemistry, HER2 FISH results, Ki67 index, pre-surgical neoadjuvant therapy, post-surgical adjuvant therapy, metastatic disease, and genetic testing.

Results: We identified 16 women with HER2-positive ILC; their characteristics are given in the table. The patients' mean age was 62 years (range, 49-75 years); all 16 patients were postmenopausal. Of the 16 patients, 4 (25%) had breast cancer history and 3 (18.8%) had atypia or malignancy in the contralateral breast. Among patients for whom the pertinent data were available, 12 of 15 (80.0%) had concurrent atypia or malignancy in the ipsilateral breast; 10 of 14 (71.5%) presented with clinical stage I or II disease; and 10 of 11 (90.9%) had a pathological disease stage of pT2 or lower. Of the 16 patients, 16 (100%) had Nottingham histological grade 1 or 2 disease; 15 (93.8) had ER-positive disease, and 7 (43.8%) had PR-positive disease. Of the 15 patients for whom HER2 IHC scores were available, 8 (53.3%) had a score of 2+, and 7 (46.7%) had a score of 3+; all 16 patients were positive for HER2 amplification by FISH (HER2:CEP 17 signal ratio ranges from 2.8 to 11.63; HER2 gene copy number arranges from 6.7 to 13.7). Ten of 15 patients (66.7%) received neoadjuvant chemo and anti-HER2 therapy, and 4 of 6 patients (66.7%) had a partial or complete response (2 partial and 2 complete).

	All Patients (n=19)	
Characteristic	Number	Percent
Malignancy history		
Breast	4	25
Non-breast	0	0
None	12	75
Contralateral breast malignancy or atypia		
Yes	3	18.8
No	13	81.3
Concurrent ipsilateral malignancy or atypia		
Yes	12	80.0
No	3	20.0
Unknown	1	
Disease site		
Right	10	62.5
Left	6	37.5
ILC type		
Classic	15	93.8
Histiocytic	1	6.3
Nottingham histological grade		
1	5	31.3
2	11	68.8
3	0	0
LVI		
No	11	100
Unknown	5	
HER2 IHC score		
2+	8	53.3
3+	7	46.7
Unknown	1	
HER2 FISH result		
Positive	16	100
Negative	0	0
Estrogen receptor status		
Positive	15	93.8
Negative	1	6.3
Progesterone receptor status		
Positive	7	43.8
Low positive	3	18.8
Negative	6	37.5
Ki67 index		
<17	8	61.5
17-35	4	30.8
>35	1	7.7
Unknown	3	
Neoadjuvant therapy		
Yes	10	66.7
No	5	33.3
Unknown	1	
Neoadjuvant therapy response		
Partial	2	33.3
Complete	2	33.3
No	2	33.3
Unknown	4	
Surgery		
Bilateral partial/complete mastectomy	2	16.7
Unilateral partial/complete mastectomy	9	75.0
Other	1	8.3
Unknown	4	
Postsurgical chemoradiation		
yes	8	66.7
No	4	33.3
Unknown	4	
Hormonal treatment		

Yes	11	91.7
No	1	8.3
Unknown	4	
Genetic testing		
Positive	0	0
Negative	6	100
Unknown	10	
Clinical stage		
I	4	28.6
II	6	42.9
III	1	7.1
IV	3	21.4
Unknown	2	
Tumor size		
pT0	2	18.2
pT1	4	36.4
pT2	4	36.4
pT3	1	9.1
pT4	0	0
Unknown	5	
Node status		
Positive	1	8.3
Negative	11	91.7
Unknown	4	

Conclusions: HER2 positivity rarely occurs in classic ILC. Because histological and clinical features are not predictive of HER2 positivity, the evaluation of HER2 status should not be skipped based on histological features such as low nuclear grade. HER2-positive ILCs have high response rates to neoadjuvant systemic therapy.

189 Comparison of Three FDA Approved PD-L1 Immunohistochemistry Assays in Triple Negative Breast Carcinoma

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Disclosures: Xiao Huang: None; Qingqing Ding: None; Hua Guo: None; Dawen Sui: None; Yun Gong: None; Yun Wu: None; Hui Chen: None; Wei-Lien Billy Wang: None; Lei Huo: None

Background: Several PD-L1 immunohistochemistry (IHC) assays have been approved by the FDA as companion or complementary diagnostics in different cancer types. The scoring methods of PD-L1 staining vary depending on the assay and the tumor type. Data on the comparison of PD-L1 assays in triple negative breast cancer are scant. Here, we compare the 28-8 Dako assay, 22C3 Dako assay and SP142 Ventana assay in triple negative breast cancer.

Design: IHC staining for PD-L1 was performed on tissue microarrays constructed from primary triple negative breast cancer resection specimens of 101 patients diagnosed between 2004 and 2016 and treated in our institution. All patients received standard therapy without PD-1/PD-L1 inhibitors. Percent PD-L1 expression in tumor-infiltrating immune cells (IC) was assessed as the proportion of tumor area occupied by PD-L1-positive immune cells of any intensity. Percent PD-L1 expression in invasive carcinoma cells (TC) was calculated as the number of viable invasive carcinoma cells showing membranous staining of any intensity divided by the total number of viable invasive carcinoma cells. Percent PD-L1 expression in immune cells and invasive carcinoma cells (ICTC) was calculated as the number of PD-L1 staining cells divided by the total number of viable invasive tumor cells. 1% or greater was considered positive. Cohen's kappa coefficient (κ) was assessed for agreement between the assays.

Results: Results of staining using 1% as cutoff are shown in Figure 1. Shown in Table 1, between 28-8 and 22C3, there was almost perfect agreement for IC, and substantial agreement for TC and ICTC. SP142 showed substantial agreement with 22C3 for ICTC and IC, and moderate agreement with 22C3 for TC and with 28-8 for all three scores. When all three assays were considered, disagreement was found in 15% (15/101) of case for TC, 24% (24/99) for ICTC and 22.4% (22/98) for IC. After the negative cases by all assays were excluded, there appeared to be some differences in the extent of staining with the assays, with SP142 showing a tendency to stain less than the others, especially when tumor cells were included in the scores (Figure 2).

Factors	Kappa coefficient	Standard error	95% Confidence interval	
			Low	High
TC (n=101)				
28-8 vs 22C3	0.7469	0.0906	0.5694	0.9244
SP142 vs 28-8	0.4511	0.1243	0.2074	0.6948
SP142 vs 22C3	0.4276	0.1368	0.1594	0.6958
ICTC (n=99)				
28-8 vs 22C3	0.7675	0.0653	0.6394	0.8955
SP142 vs 28-8	0.5758	0.0831	0.4129	0.7387
SP142 vs 22C3	0.6664	0.0789	0.5118	0.8209
IC (n=98)				
28-8 vs 22C3	0.8145	0.0627	0.6917	0.9373
SP142 vs 28-8	0.5762	0.0886	0.4025	0.7498
SP142 vs 22C3	0.6159	0.0866	0.4462	0.7856
Kappa interpretation: <0 No agreement; 0.0-0.20 Slight agreement; 0.21-0.40 Fair agreement; 0.61-0.80 Substantial agreement; 0.81-1.00 Almost perfect agreement				

Figure 1 - 189

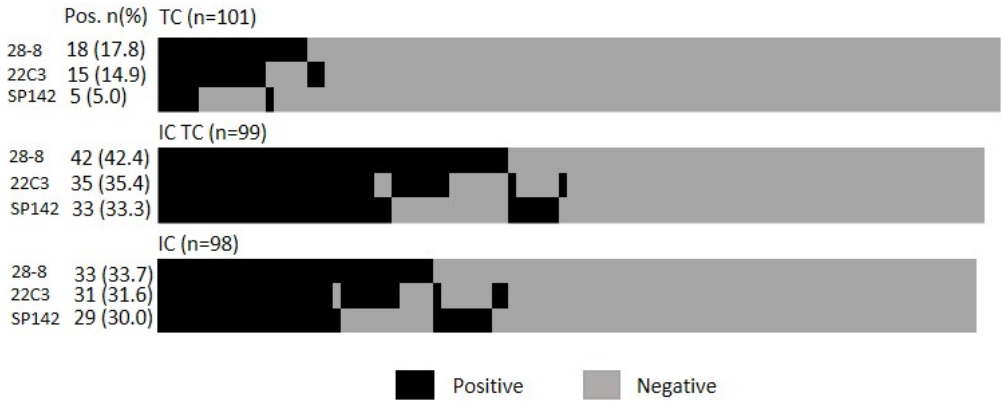

Figure 1. Staining results using 1% cutoff

Figure 2 - 189

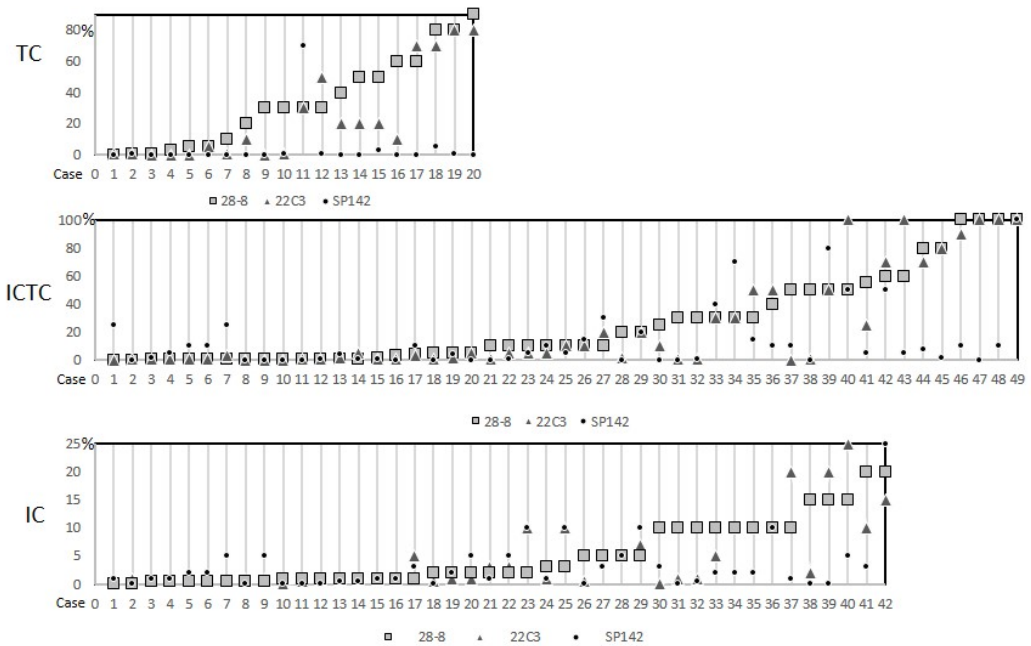

Figure 2. Percent PD-L1 staining excluding negative cases by all assays.

Conclusions: 28-8, 22C3 and SP142 showed descending order of sensitivity for detecting PD-L1 expression in triple negative breast cancer. There were better agreements between 28-8 and 22C3 than between SP142 and another assay. The clinical significance of different staining results generated from these assays requires further investigation in large clinical trials.

190 High Density of Tumor Infiltrating Lymphocytes in Triple Negative Breast Carcinoma is an Independent Predictor of Overall Survival

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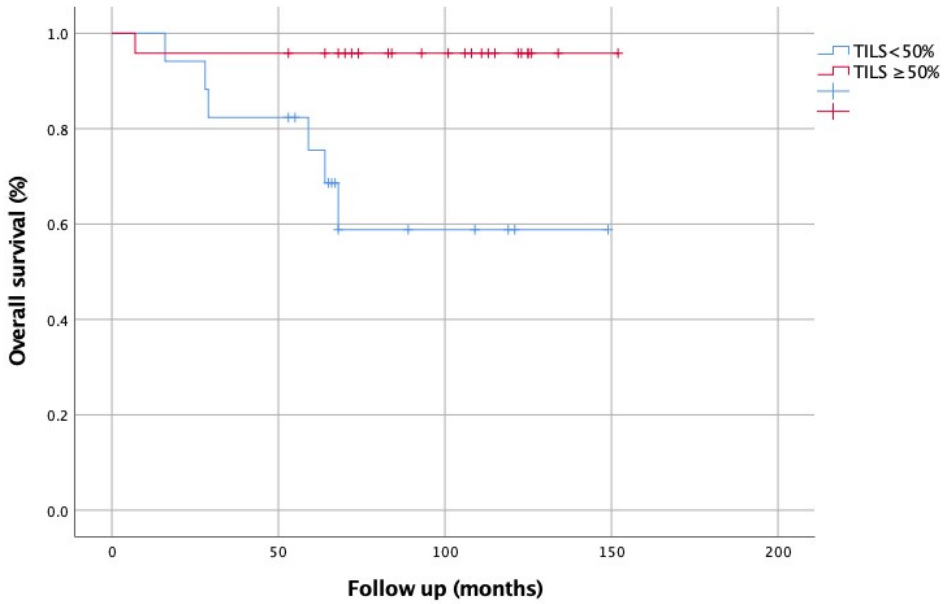
Disclosures: Mariela Huerta: None; Alexander Filatenkov: None; Yisheng Fang: None; Yan Peng: None; Helena Hwang: None; Venetia Sarode: None; Sunati Sahoo: None

Background: The role of tumor infiltrating lymphocytes (TILs) in malignancy has been studied extensively and there is robust evidence that the levels of TILs is associated with better prognosis in certain types of malignancies. The levels of TILs have been associated with better prognosis in triple negative breast cancer (TNBC) patients irrespective of treatment with chemotherapy. The methodology for evaluating TILs, however, is still evolving.

Design: We retrospectively reviewed the density of stromal TILs in 41 TNBCs, all treated with primary surgery and adjuvant chemotherapy. TILs were quantified by a pathologist blinded to the clinical outcome according to the guidelines of the International TILs Working Group 2014. We calculated the TILs volume (TILV) using an existing formula: TILV = % tumor stroma × % stromal TILs. We classified cases as TILs poor (<50%) or rich (>50%), and TILV as low (<1300) or high (>1300). We also noted the presence of any lymphoid follicles. The difference between rates of overall survival was determined using the log-rank test and Kaplan-Meier survival plots.

Results: The median overall survival (OS) for the cohort was 86 months (range, 7-152 months). The TIL-rich group had significantly better OS rates than those with TIL-poor tumors (p= 0.007). A TILV of more than 1300 correlated with better overall survival (p= 0.048). Although the presence of lymphoid follicles were more frequent in patients who had better OS, it was not statistically significant. In multivariate analysis, only the density of TILs was associated with better OS (p=0.02). TILV volume did not show a strong association with OS (p=0.051). Patients age, lymph node status, and tumor size did not show any statistical difference.

Figure 1 - 190



Conclusions: In this cohort of TNBC treated with adjuvant chemotherapy, the density of TILs emerged as a strong predictor of overall survival. We did not find any additional benefit of estimating TIL volume. This study highlights the importance of quantifying TILs routinely in pretreatment core biopsy specimens of TNBC.

191 Beta-Catenin: Use and Interpretation in the Differential Diagnosis of Fibromatosis of the Breast

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Disclosures: Sarah Hugar: None; Rohit Bhargava: None; Beth Clark: None

Background: Fibromatosis of the breast is uncommon and may be locally aggressive. Evaluation for nuclear expression of beta-catenin may aid in distinguishing fibromatosis from entities in the differential diagnosis and in evaluating resection margins, however systematic studies of beta-catenin expression in other entities in the differential have not been done. Our aim was to evaluate the staining pattern of beta-catenin in scars (both recent and remote) and myofibroblastomas, and to compare this to fibromatosis of the breast.

Design: Following IRB approval, 28 cases of fibromatosis from 22 patients, 29 remote mastectomy scars from 16 patients, 21 recent core biopsy scars from 20 patients undergoing excision of flat epithelial atypia, and 14 myofibroblastomas were retrieved from the Anatomic Pathology information system (CoPath) and selected based on slide availability. Immunohistochemistry for beta-catenin was performed on representative formalin-fixed, paraffin-embedded tissue sections (B-Catenin-1, 1:250 dilution, Dako). Cases were reviewed by two pathologists (BZC, SBH) for diagnosis and beta-catenin staining. The percent of lesional cells and stain intensity (0=absent, 1=weak, 2=strong) for nuclear, cytoplasmic, and membranous staining was recorded. Presence of granular peri-nuclear staining was noted as present or absent.

Results: In 28 cases of fibromatosis, nuclear beta-catenin staining was observed in all cases and was typically strong (27/28) and diffuse (mean 85% of lesional cells). Most remote scars (20/29), recent scars (20/21), and myofibroblastomas (13/14) were negative for nuclear beta-catenin staining. 10 of 50 scar cases showed some nuclear beta-catenin staining, but it was typically weak (8/10) and focal (less than or = 10% in 9/10). 1 myofibroblastoma showed weak and focal (10%) nuclear beta-catenin staining (Figure 1). For all diagnoses, a minority of cases showed granular cytoplasmic and peri-nuclear staining that can be misinterpreted as nuclear staining.

Figure 1 - 191

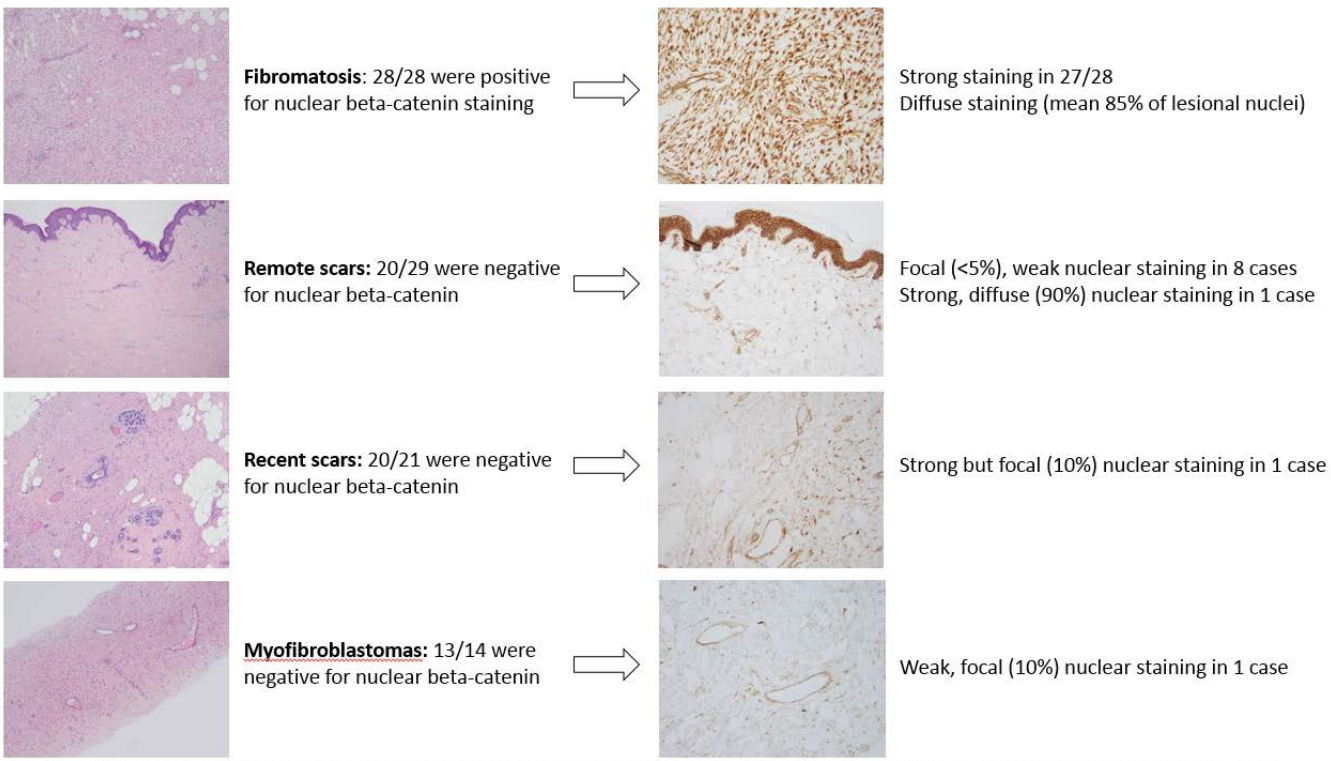

Figure 1. Summary of nuclear beta-catenin staining results in fibromatosis, remote scars, recent scars, and myofibroblastomas of the breast. In 28 cases of fibromatosis, nuclear beta-catenin staining was observed in all cases and was typically strong (27/28) and diffuse (mean 85% of lesional cells). Most remote scars (20/29), recent scars (20/21), and myofibroblastomas (13/14) were negative for nuclear beta-catenin staining. 10 of 50 scar cases showed some nuclear beta-catenin staining, but it was typically weak (8/10) and focal (</=10% in 9/10). 1 myofibroblastoma showed weak and focal (10%) nuclear beta-catenin staining. H&E photographs taken at 4x. Beta-catenin immunohistochemistry photographs taken at 10x.

Conclusions: Evaluation of nuclear beta-catenin staining is a useful tool in the differential diagnosis of fibromatosis, and in determining margin status following re-excisions. In our study, although nuclear beta-catenin was seen in a minority of scars and myofibroblastomas, it was typically focal and weak. Granular cytoplasmic and peri-nuclear staining was seen in a minority of scar, re-excision, and myofibroblastoma cases; care must be taken to avoid over-interpreting this as true nuclear staining.

192 Hypoxia-Regulated Carbonic anhydrase IX (CAIX) Protein is an Independent Prognostic Indicator in Triple Negative Breast Cancer

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Disclosures: Jabed Iqbal: None; Clara Ong: None; Bernett Lee: None; Joe Yeong: None; Jeffrey Chun Tatt Lim: None; Aye Aye Thike: None; Puay Hoon Tan: None

Background: Triple negative breast cancer (TNBC) has an aggressive biology and hence poor prognosis. To date, there are no reliable molecular markers which can reliably predict TNBC outcome. We investigated whether carbonic anhydrase IX (CAIX), a hypoxia-inducible protein can predict progression and survival in TNBC.

Design: Immunohistochemical analysis using a CAIX monoclonal antibody was performed on tissue micro-arrays constructed from FFPE tissue from TNBC patients (n=220). CAIX mRNA was extracted from FFPE tissue and digital mRNA quantification was performed by NanoString assays. CAIX protein and mRNA levels were correlated with clinicopathologic factors and survival.

Results: CAIX protein was present in 40% of TNBCs and demonstrated significant association with both DFS (p<0.0004) and OS (p<0.0001) on univariate analysis. Increased CAIX protein levels showed strong association with tumor size (p=0.0041) and histologic grade (p=0.0003). Multivariate analysis revealed that patients with CAIX-positive TNBCs had significantly worse disease-free survival (DFS) (HR = 2.95; 95% CI 1.746–4.995; *p*= 0.0001) and overall survival (OS) (HR = 2.43; 95% CI 1.34–4.411; *p* = 0.0035), after adjusting for the effects of known prognostic factors. Comparative analysis of gene expression profiles between CAIX-low and CAIX-high groups showed that seven genes were significant in these two groups of patients: ARL1, CAIX, DDIT4, IL6ST, SETX, TUBA4A and WAS. Three genes (WAS, SETX and DDIT4) were related to DNA repair.

Conclusions: Based on our results CAIX appears to be a significant hypoxia-inducible molecular marker regulating tumor progression in TNBC. Increased CAIX protein levels are independently associated with poor survival in TNBC. CAIX expression reflects significant aggressive biological behaviour in TNBC which may be used to predict clinical outcome and identify patients in need for selective CAIX-targeted therapy.

193 Gene Expression Analysis of Modified Tumor Inflammation Signature (TIS) in Triple Negative Breast Cancer among Different Ethnic Groups

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Disclosures: Deborah Jebakumar: None; Kimberly Walker: None; Arundhati Rao: None

Background: Triple negative breast carcinomas (TNBC) are aggressive neoplasms where the immune microenvironment plays a pivotal role in influencing the disease course. Approved immuno-oncology agents are now available for immune check point-based treatments in TNBCs and identifying the immune environment of tumors can be a key to more effective utilization of the same. The aim of this study is to assess the differences between the gene expression patterns as defined by the modified version of the previously published tumor inflammatory signature (TIS) between the Caucasian (CA) and African American (AA) ethnic groups with an emphasis on defining the antigen presenting regulatory pathways.

Design: The database was searched and 84 patients (42 AA and 42CA) with TNBC were identified. Total mRNA was extracted from the 84 TNBC specimens (Qiagen, MD). mRNA expressions of 785 breast cancer-related genes in 42 CA and 42 AA individuals were quantitated and analyzed using nSolver Analysis Software (V2.5). Of these, 18 genes including 5 antigen presenting cell abundance genes, 2 T cell/NK cell abundance genes, 6 interferon activity genes and 5 T cell exhaustion genes as well as 3 housekeeping genes were studied. Statistical analysis was performed.

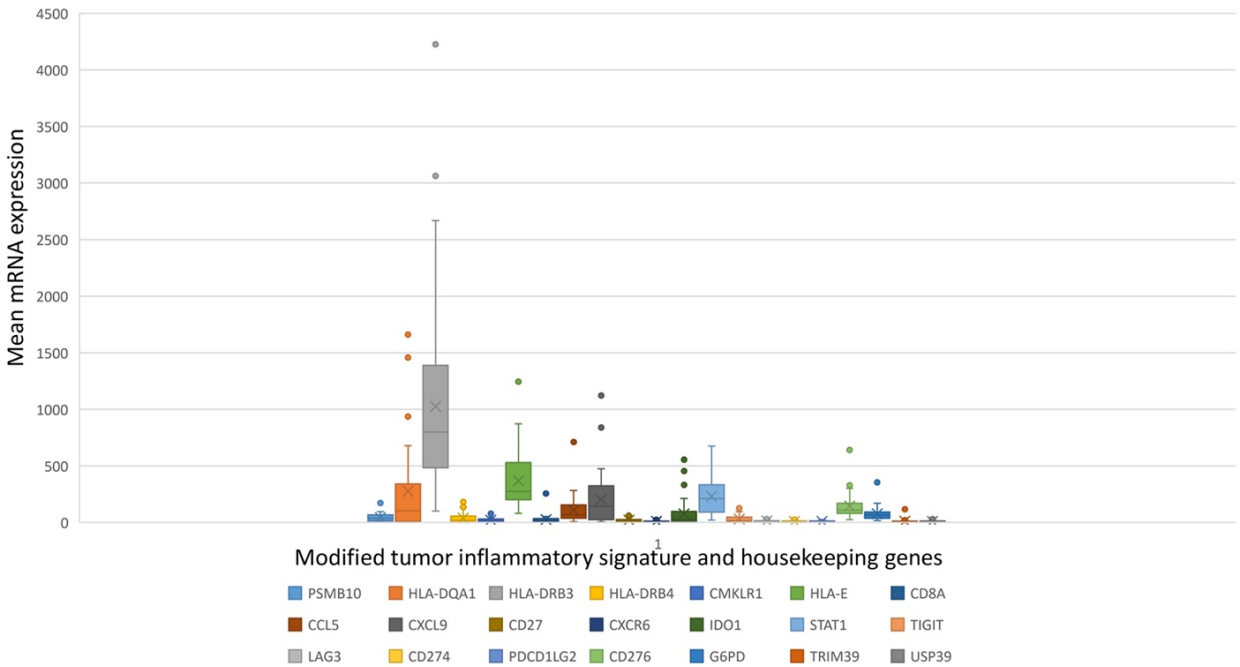
Results: All analyzed TIS genes showed higher levels of mRNA expression in the AA subset (Table 1, Figures 1 & 2). PSMB10, HLA-DRB3, HLA-E, CCL5, CD27, CXCR6, IDO1, STAT1, TIGIT, LAG3, CD274 (PD-L1) and PDCD1LG2 (PD-L2), showed statistically significant difference in expression between the two ethnic groups with p <= 0.05 (Table 1). The genes studied under the interferon activity and T cell exhaustion related TIS groups showed prominently increased mRNA expression in the AA subset in comparison to the CA subset. The differential expression of these genes correlated with an increased rate of tumor metastasis (P = 0.04) and recurrences (P = 0.003) in the AA subset. The housekeeping genes showed no differential expression as expected and served as controls.

Table 1: Modified Tumor Inflammatory Signature Genes with mean mRNA expression in the African American (AA) and Caucasian (CA) subsets

TIS biology	Gene	Mean mRNA expression (AA subset)	Mean mRNA expression (CA subset)	p Value
Antigen presenting cell abundance	PSMB10	42.23	31.70	**0.05**
	HLA-DQA1	274.47	175.58	0.07
	HLA-DRB3	1024.02	651.13	**0.007**
	HLA-DRB4	40.03	54.49	0.12
	CMKLR1	20.88	15.47	0.06
T Cell/NK Cell abundance	HLA-E	367.24	246.05	**0.003**
	CD8A	24.85	16.30	0.09
Interferon activity	CCL5	108.69	67.30	**0.02**
	CXCL9	202.09	150.38	0.17
	CD27	20.17	10.29	**0.04**
	CXCR6	9.77	7.12	**0.05**
	IDO1	76.35	19.47	**0.002**
	STAT1	230.70	162.07	**0.02**
T Cell Exhaustion	TIGIT	30.80	20.94	**0.03**
	LAG3	13.46	7.90	**0.01**
	CD274 (PD-L1)	9.05	5.79	**0.005**
	PDCD1LG2 (PD-L2)	9.58	6.85	**0.02**
	CD276	145.41	122.80	0.12
Housekeeping	G6PD	76.24	66.06	0.19
	TRIM39	11.20	7.82	0.13
	USP39	9.80	8.41	0.15

Figure 1 - 193

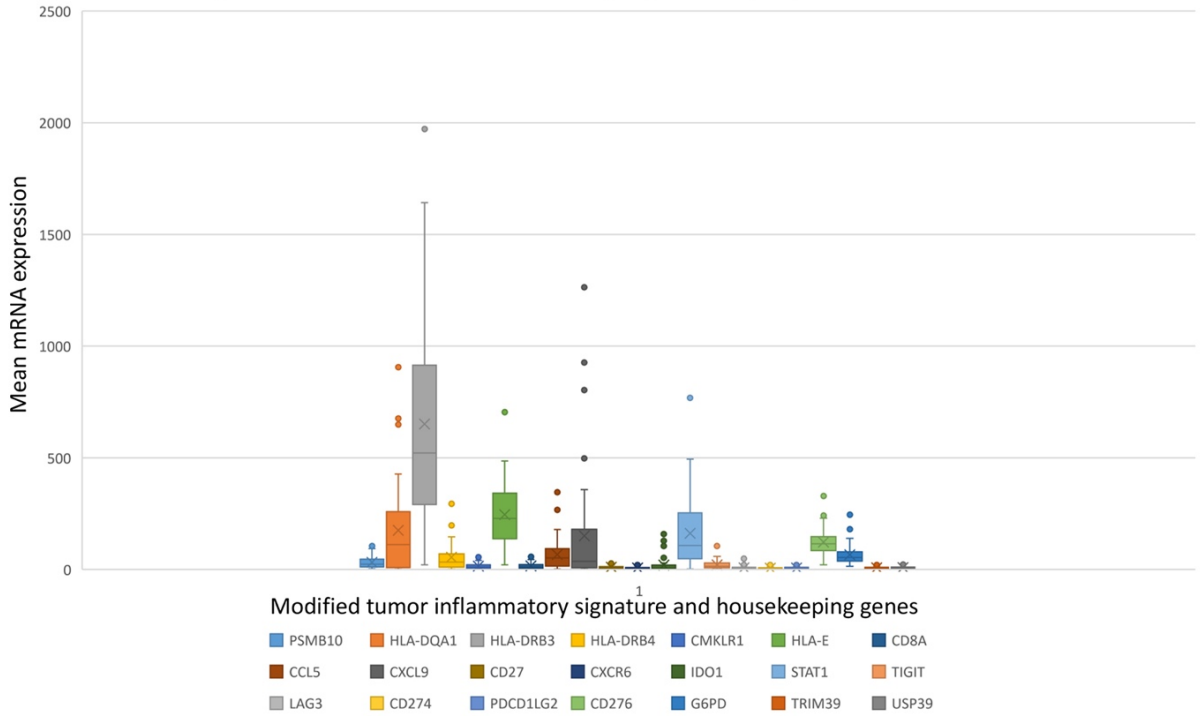
Figure 1: Modified Tumor Inflammatory Signatures (TIS) in African American Subset



Box- and whisker plot with the median of the observations(center line). The first and third quartiles are shown as boxes. The whiskers indicate the minimum and maximum value in the data set. Outliers are shown as dots.

Figure 2 - 193

Figure 2: Modified Tumor Inflammatory Signatures (TIS) in Caucasian Subset



Box- and whisker plot with the median of the observations(center line). The first and third quartiles are shown as boxes. The whiskers indicate the minimum and maximum value in the data set. Outliers are shown as dots.

Conclusions: Our study identified statistically significant differential expression of TIS genes which correlates with unfavorable outcomes. These upregulated genes could serve as useful biomarkers of outcome as well as therapeutic targets in the AA subset of TNBCs.

194 Cellular Fibroepithelial Lesion of the Breast on Core Needle Biopsy: Histologic and Radiologic Features Predictive of Upgrade to Phyllodes Tumor

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Disclosures: David Jou: None; Farhana Sharmeen: None; Alexander Brook: None; Rashmi Mehta: None; Liza Quintana: None

Background: Fibroepithelial lesions (FEL) of the breast comprise a spectrum of neoplasms spanning from fibroadenomas (FA), through benign fibroepithelial neoplasms (FEN), to phyllodes tumors (PT). Definitive categorization of cellular FELs on core needle biopsy (CNB) is challenging due to morphologic overlap. The distinction between FAs and PTs is clinically significant due to the risk of local and distant recurrence of the latter, which are currently treated with excision (EXC) with wide surgical margins. FAs are followed or enucleated. When there is histologic concern for a PT on CNB, it is our practice to call these “cellular FELs” and recommend excision. In this study, we analyzed the individual histologic and radiologic features of FELs on CNB that were upgraded to FENs and PTs on EXC as well as which combination of features was most predictive of upgrade to PTs.

Design: FELs on CNBs between 1/2012-2/2019 were identified; cases without EXC were excluded. All imaging and CNB slides were re-reviewed. Imaging and histologic features evaluated are recorded in Table 1. Spearman rank correlation and Mann-Whitney test were used to evaluate association of features with PTs. Logistic regression was used to predict upgrade to PT. Variables with association level $p<0.1$ on a multivariable logistic regression model including all variables were included in the final model.

Results: 172 cellular FELs diagnosed on CNB were identified; 111 received EXC. 72 FAs (64.8%), 14 FENs (12.6%), 18 benign PTs (16.2%), and 7 borderline PTs (6.3%) were identified. Features that were statistically significant for upgrade to PTs are bolded in Table 1. A model including lesion size, stromal cellularity, and fragmentation was able to predict upgrade to PT with area under the ROC curve of 0.88.

Characteristics Analyzed	No Upgrade (FA) n=72	Upgrade (FEN) n=14	Upgrade (PT) n=25	p value
Median age (range), years	39.3 (20-68)	46.7 (24-78)	42.3 (21-76)	0.31
Radiological Features				
BI-RADS, No. (%)				0.13
3	3 (4%)	0 (0%)	1 (4%)	
4A	55 (76%)	8 (57%)	17 (68%)	
4B	14 (20%)	5 (36%)	5 (20%)	
4C	0 (0%)	1 (7%)	1 (4%)	
5	0 (0%)	0 (0%)	1 (4%)	
Size on US, cm	**1.6**	**1.4**	**2.6**	**0.000086**
Irregular margin on US, No. (%)	15 (21%)	7 (50%)	5 (20%)	0.48
Pathological Features				
Stromal Cellularity, No. (%)				**0.00023**
Not increased/Low	66 (92%)	13 (93%)	10 (40%)	
Intermediate	6 (8%)	1 (7%)	13 (52%)	
High	0 (0%)	0 (0%)	2 (8%)	
PASH	16 (22%)	4 (29%)	5 (20%)	0.98
Stromal cell nuclear pleomorphism, No. (%)				**0.0011**
Not present/Mild	70 (97%)	13 (93%)	21 (84%)	
Moderate	2 (3%)	1 (7%)	4 (16%)	
High	0 (0%)	0 (0%)	0 (0%)	
Mitoses per 10 high-power fields (≥1)	27 (38%)	6 (43%)	13 (52%)	0.22
Irregular/ill-defined borders	9 (13%)	6 (43%)	3 (12%)	0.3
Heterogeneity	**30 (41.7%)**	**7 (50%)**	**17 (68%)**	**0.035**
Subepithelial stromal condensation	12 (17%)	2 (14%)	5 (20%)	0.8
Stromal overgrowth (10x magnification)	13 (18%)	2 (14%)	10 (40%)	0.062
Predominantly intracanalicular pattern	45 (63%)	5 (36%)	21 (84%)	0.39
Fragmentation	**20 (28%)**	**3 (21%)**	**14 (56%)**	**0.036**

Conclusions: On EXC, 35.1% of FELs on CNB were upgraded to FENs and PTs (12.6% FENs, 22.5% PTs). In our cohort, features that were independently predictive of upgrade to PTs included lesion size, stromal cellularity, stromal cell nuclear pleomorphism, heterogeneity, and fragmentation. A combination of stromal cellularity, fragmentation, and lesion size on US was highly predictive of upgrade to PTs. In our experience, a subset of each category had indistinct histologic margins which likely contributed to some FAs being categorized as cellular FEL on CNB. FELs are challenging to categorize on CNB and this study suggests that certain features and combination of features are most useful when evaluating CNB of these lesions.

195 A Multicenter Study on Pathologic Diagnosis of Breast Papillary Lesions on Core Needle Biopsy: Interobserver Variability with 2012 WHO Classification

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Background: Breast papillary lesions consist of various and heterogenous diseases ranging from benign to malignant lesions, and they have overlapping pathologic features and several different classifications, making it the most difficult diagnostic challenges in breast pathology. We aim to investigate the diagnostic agreement of papillary lesions in breast core needle biopsy specimens.

Design: Sixty papillary lesions diagnosed on breast core needle biopsy were reviewed by twenty breast pathologists from twenty institutions in Korea. The same pathologists also reviewed immunohistochemical (IHC) stains for CK5 and p63 performed in the same cases. The diagnostic agreement with or without IHC was analyzed with 2012 WHO classification and then compared to other classification systems like four-tier, three-tier, and two-tier classifications.

Results: The overall kappa coefficient was 0.20, 0.31, 0.40, 0.43 by H&E slides alone and 0.28, 0.38, 0.37, 0.63 by additional IHC stains with WHO, four-tier, three-tier, and two-tier classification systems, respectively. The interobserver agreement improved with the use of IHC in most classifications except for three-tier classification system. WHO classification increased interobserver variability in comparison with any other classifications by both H&E (k=0.20) and IHC (k=0.28) stains. The highest agreement was seen in two-tier classification by IHC stains (k=0.63). In spite of IHC stains, encapsulated/solid papillary carcinoma (E/SPC) subgroup (k=0.16) showed lower agreement than non-E/SPC subgroup (k=0.35) with WHO classification, which was similar to the results with any other classifications.

Conclusions: Although the use of IHC for CK5 and p63 increases the diagnostic agreement of papillary lesions in breast core needle biopsy specimens, WHO classification shows higher discordance rate than any other classifications. Therefore, more intensive consensus studies are imperative to improve the diagnostic agreement and categorization of these lesions with WHO classification.

196 Multiple Foci of Invasive Breast Carcinoma: Insights and Expectations Based on Biomarker Profiles

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Disclosures: Michelle Khieu: None; Paolo Cotzia: None; Farbod Darvishian: None

Background: The American Joint Committee on Cancer (AJCC) recommends the use of the largest tumor size for pathologic staging of multifocal breast cancer (MBC). However, the recently updated AJCC 8th edition now takes biologic factors such as grade, biomarker profile, and genomic panels into account for breast cancer prognostication. We sought to study MBCs at our institution and track their path to metastasis in an attempt to gain further insight into variables of aggressiveness other than traditional size.

Design: We searched for MBC cases within our database from 2010 through 2017. Age, procedure type, laterality, size, histologic grade (G), presence of metastasis (axillary or distant) and biomarker profile of the primary and metastases were recorded. The biomarker profiles

were classified as hormone receptor positive (HR+), human epidermal growth factor 2 positive (Her2+) or triple negative (TN), and deemed concordant (C) if similar or discordant (D) if dissimilar.

Results: Of 473 MBCs, 260 had at least two biomarker profiles reported. The majority of the latter patients underwent total mastectomy (67%) while 33% had partial mastectomy. Of 554 total tumor foci, 462 (93% C, 7% D) were HR+ (17% G1, 62% G2, 21% G3), 65 (66% C, 34% D) were Her2+ (34% G2, 66% G3) and 27 (74% C, 26% D) were TN (19% G2, 81% G3). There were 233 (90%) concordant cases and 27 (10%) discordant. Of the 260 cases, 12 (4.6%) discordant cases had tumor foci with the same morphology and G. Twelve of the discordant cases had axillary and/or distant metastases, 4 of which with a biomarker profile available for the metastatic tumor (Table). The metastatic biomarker profiles of these 4 cases indicate that, of the two primary foci, the focus with the largest tumor size, equal or higher G, and Her2+ status metastasized in 75%, 75% and 50% of cases, respectively.

Discordant case	T1 Size (cm), Type, Grade	T1 biomarker	T2 Size (cm), Type, Grade	T2 biomarker	Metastasis biomarker
1	2.0, Mixed, 2	HR+	0.7, IDC, 3	Her2+	HR+
2	2.0, IDC, 2	HR+	1.1, IDC, 2	Her2+	HR+
3	2.7, IDC, 3	Her2+	0.5, IDC, 2	HR+	Her2+
4	1.3, ILC, 2	HR+	0.5, IDC, 3	Her2+	Her2+

IDC = Invasive ductal carcinoma; ILC = Invasive lobular carcinoma; HR+ = Hormone receptor positive; Her2+ = Human epidermal growth factor 2 positive

Conclusions: Our data show that about 90% of patients with MBC can be expected to have the same biomarker profile in all tumor foci. However, 4.6% of cases with multiple biomarker profiles showed discordant profiles despite similar morphology and G. It can be concluded then, that a similar percentage of cases on which only one set of biomarkers were performed, actually had discordant profiles. These patients may have had additional treatment options than otherwise indicated by the single biomarker profile. In addition, our data shows that tumor G, size and biomarker profile all contribute to predicting tumor behavior, supporting the updated AJCC 8th edition.

197 Impact of the Updated American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) Guidelines on HER2 Test in Breast Cancer: Comparison of 2007, 2013 and 2018 Recommendations

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Disclosures: Min Chong Kim: None; Young Kyung Bae: None

Background: HER2 status is an important prognostic and predictive factor in breast cancer, and it should be evaluated for every invasive breast cancer (IBC) patient in current clinical practice. Since the ASCO/CAP established HER2 testing guideline in 2007, two major updates were made in 2013 and 2018. We assessed the clinical impact of the updated ASCO/CAP guidelines by comparing categorization according to the 2007, 2013, and 2018 ISH classification criteria.

Design: We collected 1183 IBC cases with both IHC and silver-enhanced ISH (SISH) results for HER2 from January 2010 to October 2013. Specimens consisted of primary breast carcinomas resected by surgical excision. Recently, in South Korea, immunohistochemistry (IHC) is the primary test for HER2, and ISH could be performed in only IHC 2+ cases. For a while, all IBC cases were referred to both IHC and ISH until October 2013. Initial HER2 status were interpreted according to 2007 guidelines by a senior breast pathologist at the time of diagnosis. The average HER2 and CEP17 signals per cell, and HER2/CEP17 ratio were obtained from pathology reports. The cases were grouped under each 2007, 2013, and 2018 ASCO/CAP criteria and compared the results each other.

Results: Compared to 2007 recommendations, 25 (2.1%) and 14 (1.2%) cases were recategorized under 2013 and 2018 updates, respectively (Figures 1 and 2). The 2013 guidelines increased positive cases (5 from equivocal, and 3 from negative) in comparison to 2007. Any specific tendency was not observed in comparison between 2007 and 2018 criteria (Figure 2). Seventeen (1.4%) cases were recategorized from 2013 to 2018 guidelines (Figure 1). Most of equivocal cases (13/14) in 2013 criteria became negative according to 2018 guidelines, and most of them (12/13) were negative according to 2007 guidelines. Among 1183 cases, 26 (2.2%) were affected by 2013 and 2018 guideline updates.

Figure 1 - 197

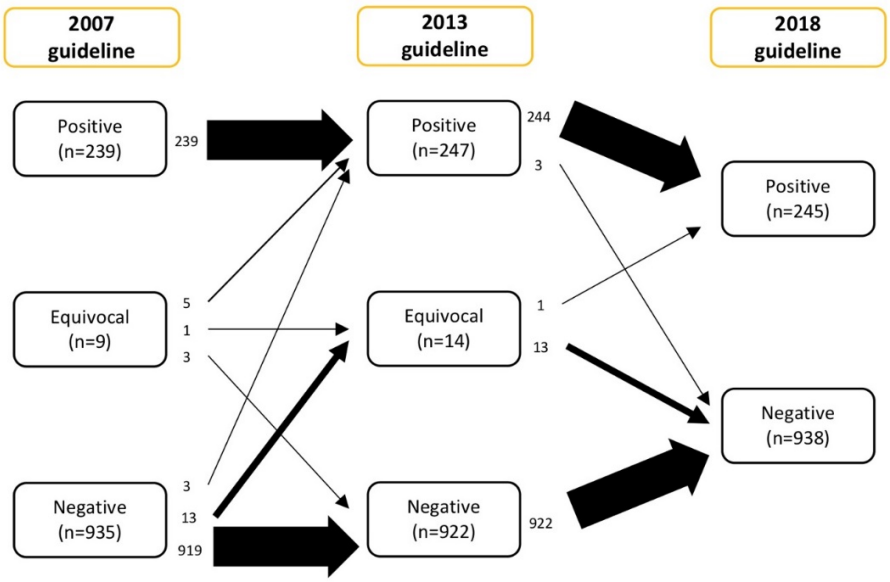
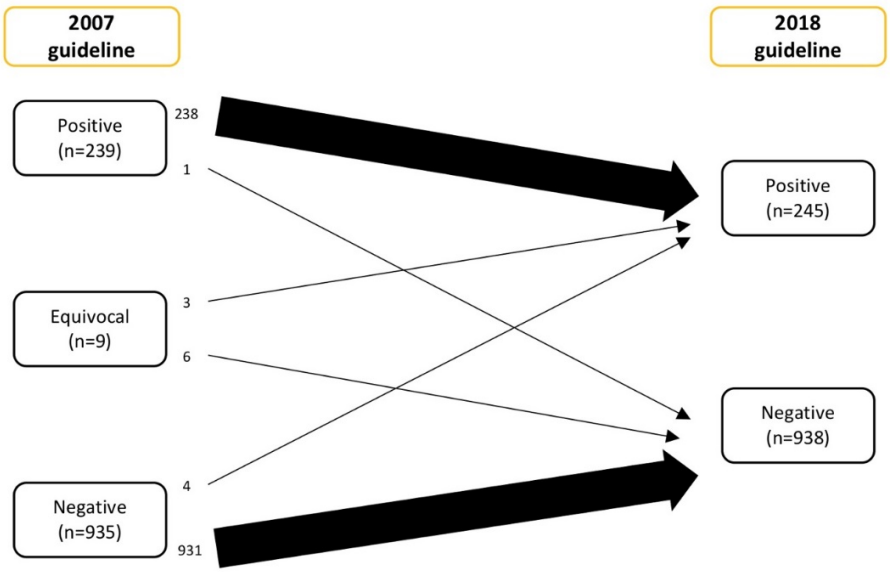


Figure 2 - 197



Conclusions: Our results showed that positive and equivocal cases were increased under 2013 guidelines, compared to 2007 guidelines. On the other hand, noticeable change in 2018 guideline is elimination of ambiguous cases by reclassification of them into HER2-negative group. We are expecting the 2018 ASCO/CAP guideline may give precise option to patients' treatment. Further studies are needed for clinical outcome of new criteria.

198 Evaluation of SOX10 Staining in Metaplastic Carcinoma and Spindle Cell Lesions of the Breast

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Disclosures: Kristina-Ana Klaric: None; Sarah Strickland: None; Tadros Atalla: None; Zuzana Kos: None

Background: Metaplastic carcinoma of the breast is a rare but aggressive cancer. A diagnostic challenge, particularly in small biopsies, is differentiating spindle cell metaplastic carcinoma from other benign and malignant spindle cell lesions of the breast. SOX10 is expressed in a subset of breast cancers, particularly triple-negative and basal-like breast cancers. We evaluated SOX10 staining in metaplastic carcinomas and other spindle cell lesions of the breast.

Design: SOX10 staining was performed on cases of metaplastic carcinoma and other spindle cell lesions of the breast (malignant phyllodes, primary breast sarcoma, fibromatosis, nodular fasciitis, myofibroblastoma, solitary fibrous tumour and inflammatory myofibroblastic tumour) identified from our institution's pathology archives from 2003–2018. Staining in ≥1% of cells was considered positive.

Results: A total of 76 cases of metaplastic carcinoma were identified, including 20 spindled, 8 mixed spindle and other mesenchymal components, 25 squamous, 18 chondroid and 5 showing other morphologies. Of the 28 cases with a spindle cell component, 10 showed low/intermediate-grade monotonous spindle cells and 18 showed frankly malignant high-grade sarcomatous cells. SOX10 was positive in 34 (45%) metaplastic carcinomas with differing frequency across subtypes – staining 96% of chondroid and 26% of squamous cases. Only 14% (4/28) of spindle cell metaplastic carcinomas stained positive with SOX10, all 4 cases showing high-grade sarcomatous spindle cells (22%). No SOX10 staining was seen in malignant phyllodes tumours (0/11) or primary breast sarcomas (0/13). None of the lower grade spindle cell metaplastic carcinomas stained for SOX10, while a small proportion of nodular fasciitis (1/4) and fibromatosis (3/25) cases showed weak focal positivity (1% staining). SOX10 was negative in all cases of myofibroblastoma (0/11), solitary fibrous tumour (0/3), and inflammatory myofibroblastic tumour (0/2).

Conclusions: SOX10 stains most metaplastic carcinomas with chondroid (matrix-producing) features but shows minimal expression in carcinomas with spindle-cell morphology. SOX10 expression in a high-grade sarcomatous lesion supports metaplastic carcinoma over malignant phyllodes tumour or primary sarcoma, but is expressed in only a minority of cases. There is no role for SOX10 in differentiating metaplastic spindle cell carcinoma from the other spindle cell lesions of the breast.

199 Morphologic Variants of Lobular Carcinoma In Situ (LCIS) Diagnosed on Core Needle Biopsy: Clinicopathologic Features and Findings at Follow-Up Excision

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Disclosures: M Gabriela Kuba: None; Melissa Murray: None; Kristen Coffey: None; Catarina Calle: None; Monica Morrow: *Speaker*, Genomic Health; Edi Brogi: None

Background: The morphologic spectrum of LCIS includes classic type (CLCIS) and rare variants, namely florid (FLCIS) and pleomorphic (PLCIS). The behavior and management of FLCIS and PLCIS are not fully characterized. We assessed the clinicopathologic features and upgrade rate at excision (EXC) of variant LCIS diagnosed on core needle biopsy (CNB) at our center.

Design: We searched our pathology database for inhouse CNBs obtained 1/2000-6/2019 with a diagnosis of "carcinoma in situ with ductal and lobular features", or LCIS and "pleomorphic", "massive acinar expansion" and/or "necrosis". Patients (pts) with concurrent ipsilateral (micro)invasive carcinoma, DCIS, ADH, no excision, or no available slides were excluded. Upon slide review we recorded CLCIS, PLCIS (nuclear atypia akin to high grade DCIS), FLCIS (classic LCIS cytomorphology with little/no intervening stroma between markedly distended acini and/or >40 cells in diameter), LCIS with pleomorphic features (LCIS-PF; cytomorphology between CLCIS type B cells and PLCIS), apocrine features, necrosis, and calcifications. A radiologist reviewed all pertinent imaging studies. Upgrade was defined as (micro)invasive carcinoma or DCIS at EXC. The IRB approved the study.

Results: The patient cohort consisted of 33 women (median age 61 years, range 45-79); 15 (45%) pts had history of breast cancer (BC), and 3 of CLCIS. There were a total of 34 CNBs: 8 (23%) had PLCIS, 22 (65%) FLCIS and 4 (12%) LCIS-PF. Apocrine features were seen in 63% PLCIS and 23% FLCIS. Table 1 summarizes clinicopathologic features and findings at EXC. All cases were radiologic-pathologic concordant except one (imaging: irregular mass; CNB: apocrine FLCIS; EXC: no upgrade). The upgrade rate of PLCIS and FLCIS combined was 20%. The CNBs with upgrade had 2 PLCIS+necrosis, and 4 FLCIS+necrosis. Menopausal status, BC history, calcifications, and apocrine features did not correlate with upgrade, but necrosis showed a positive trend (p=0.06, x^2 test). At follow-up one pt (CNB FLCIS, EXC no upgrade, no radiation or hormonal therapy) recurred with microinvasion 24 months (mo) later. Another pt (CNB PLCIS, EXC microinvasion, no radiation or hormonal therapy) recurred with invasive carcinoma at 41 mo.

Table 1. Clinicopathologic features and findings on core needle biopsy and excision

CNB findings		Median age	Imaging findings				Upgrade at excision	Mastectomy (M) vs Lumpectomy (L)	Radiation therapy	Endocrine therapy
Diagnosis	N		Calcifications	NME	Mass	Architectural distortion				
All cases[a]	34	61 (45-79)	23	7	3	1	6 (18%)	M=4 L=30	4/34	16/34
Variant LCIS (PLCIS and FLCIS)	30	61 (45-79)	22	4	3	1	6 (20%)	M=4 L=26	4/30	15/30
PLCIS										
with necrosis[b]	5	64 (45-75)	4	0	1	0	2[f]	M=0 L=5	1/5	2/5
without necrosis[c]	3	60 (48-69)	2	1	0	0	0	M=2 L=1	0/3	1/3
total	8	62.5 (45-75)	6	1	1	0	2 (25%)	M=2 L=6	1/8	3/8
FLCIS										
with necrosis[d]	16	61 (48-79)	13	1	2	0	4[g]	M=2 L=14	3/16	5/16
without necrosis[e]	6	61.5 (54-74)	3	2	0	1	0	M=0 L=6	0/6	1/6
total	22	61 (48-79)	16	3	2	1	4 (18%)	M=2 L=20	3/22	6/22
LCISPF	4	52.5 (49-73)	1	3	0	0	0 (0%)	M=0 L=4	0/4	1/4

[a]All cases were e-cadherin negative on IHC

[b]1 apocrine PLCIS, 1 case with FLCIS, and 2 cases with apocrine PLCIS+apocrine FLCIS

[c]1 case with apocrine PLCIS and 1 with apocrine PLCIS+apocrine FLCIS

[d]3 apocrine FLCIS

[e]2 apocrine FLCIS

[f] One ILC (0.5 cm, ER+/PR+/HER2-), one LCIS with microinvasion (hormone receptor status not available)

[g] One ILC (0.4 cm, ER+/PR+/HER2-) and 3 LCIS with microinvasion (1 ER+,PR+/HER2+, 1 ER+/PR-/HER2-, 1 hormone status not available)

Conclusions: We found apocrine features in 23% FLCIS, previously reported only in PLCIS. The upgrade rate of variant LCIS was 20%. At follow-up, 2 pts developed micro and/or invasive carcinoma. Our findings add to the limited follow-up data on variant LCIS with possible management implications.

200 The Impact of MYC Amplification on Clinicopathologic Features and Prognosis of Radiation-Associated Angiosarcomas of the Breast

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Disclosures: M Gabriela Kuba: None; Bin Xu: None; Cristina Antonescu: None

Background: Radiation-associated angiosarcomas of the breast (RT-AS) are rare tumors with overall poor prognosis. *MYC* amplification represents the genetic hallmark of these tumors and is often used as a diagnostic marker to distinguish from an atypical vascular lesion. However, a small subset of RT-AS lacks *MYC* amplifications, which might be difficult to diagnose and might be associated with a distinct pathogenesis. In this study we sought to examine the impact of *MYC* gene amplification as detected by fluorescence in situ hybridization (FISH) and/or next generation sequencing (NGS) on clinicopathologic features and outcome in a large cohort of RT-AS managed at our institution.

Design: The pathology database was searched for cases of RT-AS diagnosed between 1/1998 and 8/2019. All patients had been previously treated with radiotherapy for breast cancer (BC). All cases were tested for *MYC* amplification by FISH or NGS. Clinicopathologic and follow-up data were obtained from the medical records. Fisher's exact test was used for comparison of *MYC* amplified and not amplified groups. Log rank test and Cox proportional hazard model were used for outcome analysis.

Results: 81 women were selected, with a median age at diagnosis of 69 years (range 48-95) and a median interval from BC to RT-AS diagnosis of 7.5 years (range 3-25). Clinical presentation included skin erythema/bruising +/- thickening (61%), palpable masses (34%) or papules (3%). Initial surgery consisted of mastectomy for all patients status-post lumpectomy for BC (n=71), and wide local excision in those with prior mastectomy (n=6). Two patients had extensive breast involvement at diagnosis and did not undergo surgery. All except 2 RT-AS (97%) were high grade, 12 of 65 (19%) showed epithelioid features, 19 of 64 (30%) tumor >5 cm, and 7 of 73 (10%) had positive margins at initial surgery. Eight (10%) cases lacked *MYC* amplification. Median follow up was 22 months (range 0-231); 5-year overall survival (OS) 51%. On univariate analysis, positive margin (p=0.002) and *MYC* amplification (p=0.026) was associated with decreased OS. On multivariate analysis positive margin was the only independent adverse factor associated with worse OS (HR 3.72, 95% CI 1.38-10.02, p=0.009).

Conclusions: Lack of *MYC* amplification is seen in 10% of RT-AS and does not exclude a diagnosis of malignancy. RT-AS have an overall poor OS, with positive margin status and *MYC* amplification being adverse factors. Histologically low grade RT-AS were only seen in *MYC*-non-amplified group.

201 Validation of a New Method to Evaluate Mitotic Activity in Core Biopsies with Invasive Breast Carcinoma

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Disclosures: Timothy Law: None; Whayoung Lee: None; Julio Ibarra: None

Background: Histologic grade measured by the Nottingham Histologic Grading System (NHGS) is a strong prognostic predictor in patients with breast carcinoma. Mitotic activity, one of the components of the NHGS, is scored based on the number of mitoses seen in ten contiguous high-power fields (HPFs). A common pitfall encountered in limited biopsy specimens is the inability to find ten HPFs of the tumor at the tumor edge. Therefore, we have developed a method to grade mitotic activity in core biopsies by counting the highest number of mitoses in one HPF at the tumor edge where the mitotic activity is the highest. We propose a score of 1, 2, or 3, if we find 0-1, 2, or >= 3 mitoses in one HPF, respectively.

Design: A total of 133 cases of breast core biopsy, followed by a partial or complete mastectomy with a diagnosis of invasive breast carcinoma were reviewed. Cases with tumors less than ten HPFs, microinvasive carcinoma, and with neoadjuvant treatment were excluded. Each investigator, utilizing a microscope with field diameter of 0.55 mm, performed a blinded mitotic count in one and ten HPFs in each core biopsy and ten HPFs in each accompanying resection specimen and then converted to mitotic scores. For the ten HPF counts, we followed the College of American Pathologists (CAP) recommendations: 0-8 mitoses 1 point, 9-17 mitoses 2 points, and >= 18 mitoses 3 points. The correlation between the scores in the biopsy and excision specimen was measured by the Pearson correlation coefficient (r).

Results: The r was calculated as follows: mitotic score in one and ten HPFs in each core biopsy; r=0.705, mitotic score in ten HPFs in each core biopsy and ten HPFs in the accompanying resection specimen; r=0.678, and mitotic score in one HPF in each core biopsy and ten HPFs in the accompanying resection specimen; r=0.642. The concordance rates between each group were 84.2%, 83.5%, and 80.5% respectively. All r values exhibited a significant correlation with p-value <0.01.

Conclusions: Our results demonstrate significant correlation and concordance rate between one HPF and ten HPFs mitotic score in each core biopsy. The correlation and concordance rate of the mitotic score between one HPF in the core biopsy and ten HPFs in the excision is comparable to that of ten HPFs in the core biopsy and excision. These results show our proposed method may be utilized in conjunction with conventional mitotic scoring methodologies, especially when a small amount of tissue is available for grading in core biopsies.

202 Changes in Hormone Receptors, HER2, and Molecular Subtype between Primary Breast Carcinomas and Metastatic Carcinomas to the Lung Treated with Neoadjuvant or Adjuvant Chemotherapy

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Disclosures: Ho-chang Lee: None; Seung Geun Song: None; Ji Hye Moon: None; Jin Woo Park: None; Han Suk Ryu: None; In Ae Park: None

Background: Estrogen receptor (ER), progesterone receptor (PR), and HER2 are important markers of breast carcinoma. Differences of immunohistochemical (IHC) markers among primary breast carcinoma (PBC) specimens before chemotherapy and metastatic carcinoma specimens to the lung (MCL) can affect the treatment plans and prognoses of patients.

Design: One hundred and six cases, 34 neoadjuvant chemotherapy (NACT) and 72 adjuvant chemotherapy (ADCT), were selected in which paraffin blocks of PBC and MCL were available. NACT and ADCT were consisted of docetaxel and doxorubicin, and hormone and/or trastuzumab therapy were done according to expression profiles of PBC. Expression of ER, PR, and HER2 was assayed in PBC and MCL. Cutoff values of ER and PR positivity were both 10% and strong membranous staining by immunohistochemistry and/or amplification by fluorescence in situ hybridization was considered as positive for HER2. McNemar test was used for the changes of the markers. The analyses were also compared between two groups.

Results: Changes of ER, PR, and HER2 expression were shown in 5, 20, and 5 cases, respectively. No cases which two or more markers were changed simultaneously were found. There was a significant loss of PR expression between PBC and MCL in all cases (p=0.0008) whereas there was a tendency of gain of ER expression (p=0.0736). Loss of PR expression was prominent in ADCT group (p=0.0060) but not in NACT group (p=0.1336). Changes of HER2 were not statically significant (p=0.3711). Subtype was changed in seven of 98 cases (7.14%) between PBC and MCL and the changes were not statistically significant (p=0.2276). Four out of 16 HER2-positive subtype cancers in PBC were changed into two luminal and two triple-negative cancers after chemotherapy, which was due to loss of HER2 expression after trastuzumab treatment.

Conclusions: IHC studies for ER, PR, HER2 should not only be assessed in the biopsy specimens of PBC, but also in the MCL specimen after NACT or ADCT for establishing the further treatment plans. Especially, HER2 expression and/or amplification should be assayed because the expression can be lost after trastuzumab therapy.

203 The Significance of HER2 Protein Overexpression in Ductal Carcinoma In Situ of Breast: A Single Institutional Experience

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Disclosures: Mariah Leivo: None; Oluwole Fadare: None; Farnaz Hasteh: None; Somaye Zare: None

Background: HER2/neu aberrations represent an established negative prognostic factor in invasive breast cancer; however, its prognostic significance in DCIS is not known. Here, we examined HER2/neu status in DCIS to assess its clinicopathologic associations and prognostic value.

Design: HER2/neu status was evaluated in 380 patients with a diagnosis of pure DCIS on core biopsy who subsequently underwent excision at our institution. HER2/neu status was assessed using immunohistochemical staining and was scored using ASCO/CAP criteria.

Results: For the entire 380-case cohort, 10%, 32.2%, and 47.8% were low grade, intermediate grade, and high grade, respectively. The extent of disease ranged from 0.1 cm to 14 cm (mean: 2.5 cm). The subsequent excisions revealed microinvasion in 21 (5.5%) of the 380 cases, 10 (47.6%) of which were HER2/neu-positive. Nodal metastasis were identified in 2.5% of the 155 cases in which lymph node sampling was performed and 50% of the node-positive cases were HER2/neu-positive. HER2/neu protein was over-expressed at the 3+ level in 31.5% of all cases. HER2/neu positive DCIS was associated with higher nuclear grade (p<0.0001), presence of comedonecrosis (p<0.0001), larger extent of disease, and the presence of microinvasion in subsequent resection (p<0.0001). On follow-up, 32.8% of HER2/neu-positive cases were associated with a recurrence as compared with 30.1% of HER2-negative cases (p=.80).

Conclusions: In our study cohort, the frequency of HER2/neu positivity in DCIS (31.5%) was higher than historically reported data for invasive carcinomas. HER2/neu positive DCIS was associated with features of poor prognosis including higher nuclear grade, more extensive disease, and the presence of microinvasion. No statistically significant increase in risk of recurrence was identified in HER2/neu positive DCIS.

204 miR-454 Reduces Sensitivity of ER-Positive Breast Cancer Cells to Tamoxifen by Simultaneously Targeting at RUNX3 and ERα

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Disclosures: Chenxi Xiang: None

Background: Tamoxifen is a widely used endocrine therapy agent for the treatment of ER-positive breast cancer. However, tamoxifen resistance developed despite the initial response of patients. Activation of multiple cancer-promoting signaling pathways, the acquisition of estrogent independent ER signaling and also breast cancer cells gaining stem-cell like traits can all lead to tamoxifen resistance. miR-454 is a miRNA that is highly expressed in a variety of tumors, but its functional studies in breast cancer remains few. This study aimes at investigating whether miR-454 could regulate tamoxifen sensitivity of ER-positive breast cancer cells and the mechanisms underpinning the phenomenon.

Design: The expression levels of miR-454 were studied by qPCR in normal breast epithelial cell line MCF-10A, ER positive breast cancer cell line MCF-7, T47D, MDA-MB-231 and BT-474. The effect of miR-454 on cell survival and apoptosis after tamoxifen treatment was studied using CCK8 method and flow cytometry. We predicted the targets of miR-454 on miRanda, Targetsacn and miRDB. A GO analysis was performed based on the target genes shared. The combining of miR-454 to its targets was verified using the luciferase reporter assay and WB assay. The regulation of Wnt signaling pathway by miR-454 was studied using TOP-Flash system, immunofluorescence and WB assay. The regulation of miR-454 on the stem-like traits of breast cancer cells was studied using 3D tumor sphere formation assay, flow cytometry and WB assay.

Results: miR-454 was high expressed in ER-positive breast cancer cells, but low expressed in MCF-10A and ER-negative breast cancer cells (1A). miR-454 significantly reduced the sensitivity to tamoxifen of ER-positive breast cancer cells and reduced tamoxifen-induced apoptosis in ER-positive breast cancer cells (1B, 1C). The targets of miR-454 were mainly enriched in the Wnt signaling pathway (2A). Luciferase reporter assay and Western blotting assay confirmed that miR-454 could target at RUNX3 and ERα (2B, 2C). TOP-Flash assay revealed that miR-454 could regulate Wnt signaling (2D). At last, miR-454 could significantly promote the stem-like traits of breast cancer cells (2E).

Figure 1 - 204

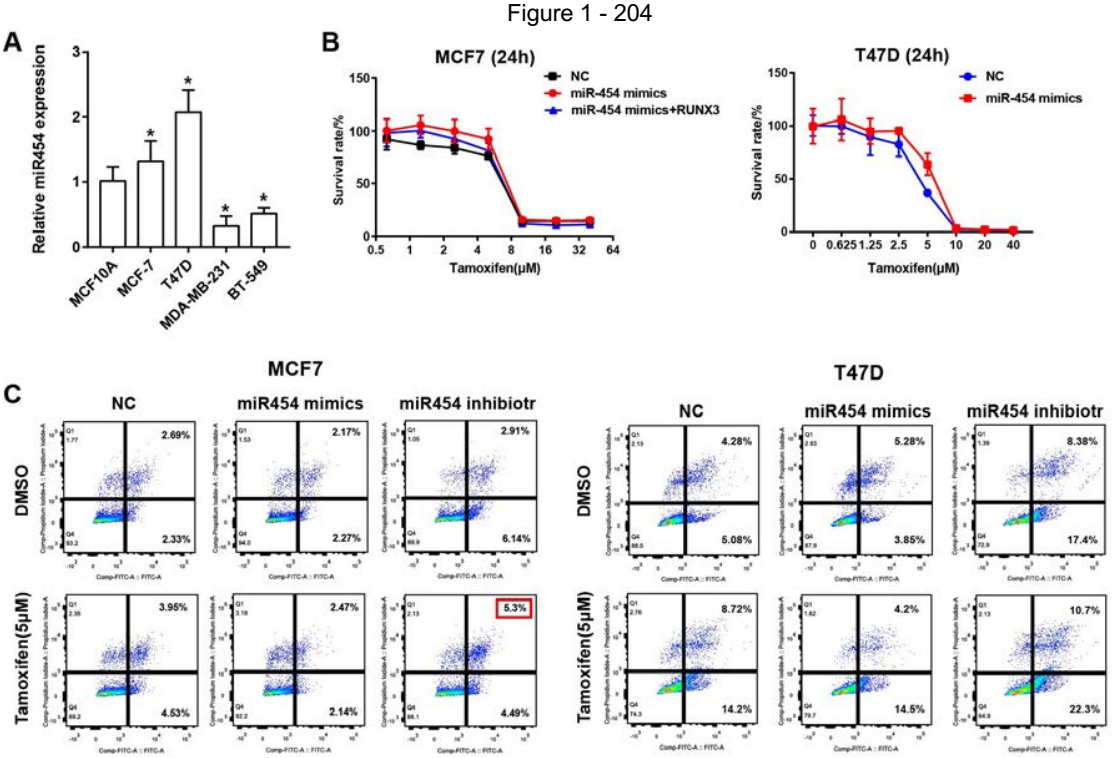
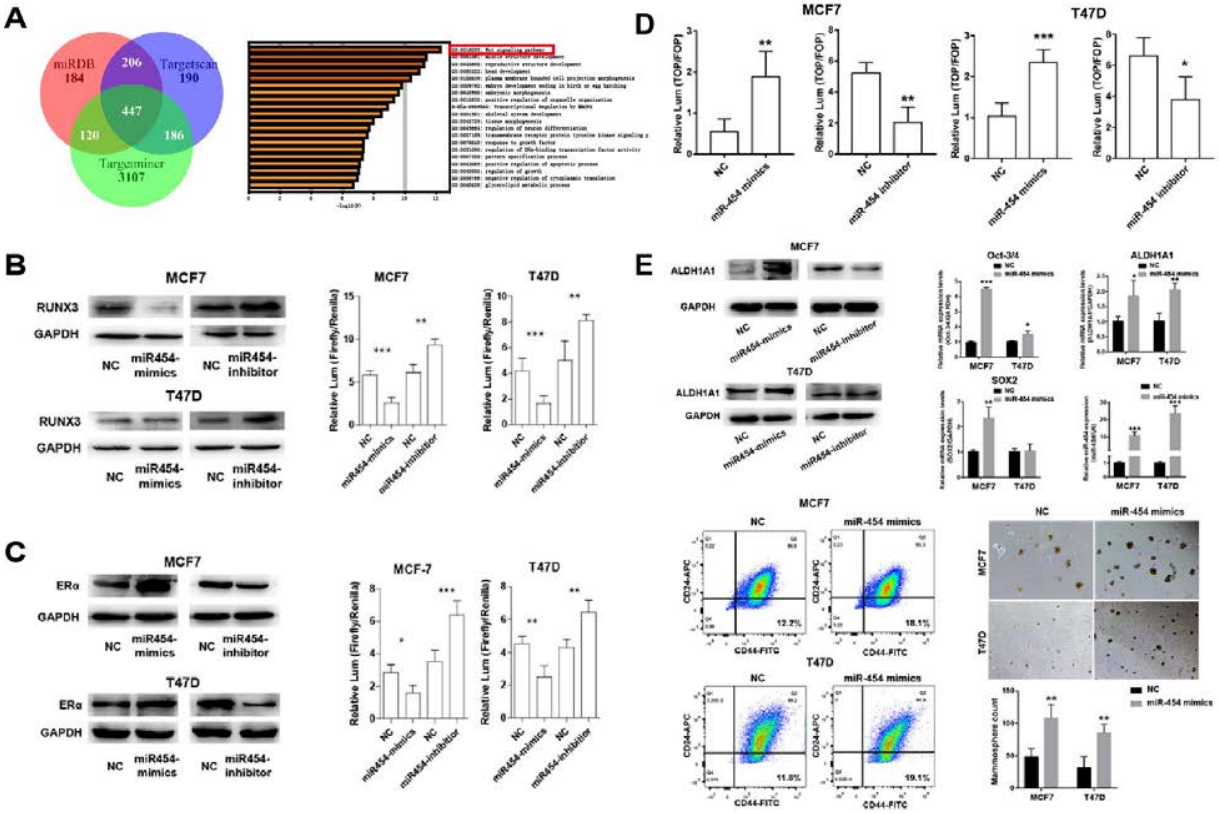


Figure 2 - 204



Conclusions: miR-454 is mainly expressed in ER-positive breast cancer cells. And miR-454 can target at RUNX3 to activate Wnt signaling pathway and promote stem-cell like traits of breast cancer cells. At the same time, it can directly target at ER to suppress its expression and inhibit the sensitivity of breast cancer cells to tamoxifen.

205 Time-Dependent and Non-Time Dependent Prognostic Factors for Invasive Breast Cancer

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Disclosures: Xulei Liu: None; Lanjing Zhang: *Employee*, Celgene; *Stock Ownership*, Celgene

Background: Accurate modelling of invasive breast cancer (IBC) survival helps improve treatment and prognostication. Commonly used Cox survival model is valid only when the assumption of constant hazard ratio (HR) is met (i.e. HR does not change as followup time changes). But it is rarely studied in prior works. Therefore, we examined the assumption of constant HR in common prognostic factors of IBC.

Design: We used the Surveillance, Epidemiology and End Results (SEER)-18 database to select women aged 20+ years with the diagnosis of primary IBC in 2004, who were followed up for at least 2 months and until 2014. Cox proportional hazards models were performed and the constant HR assumptions were examined.

Results: We included 34,013 patients, 64% of whom had ER+PR+ IBC, 73.7% had invasive ductal carcinoma (IDC), 95% had surgery, 46% had chemotherapy, 55% had radiotherapy, and 73% with menopause. Approximately 33% (11388/34013) of patients died from any causes, and 16% (5412/34013) from IBC.

In both all-cause and IBC-specific mortality analyses, menopause, black, histologic grade 3, pT3 or pT4, pN+, pM+, and ER+PR- were associated with a constant higher risk of mortality throughout the study period compared to their reference groups, respectively; other race, surgery, and chemotherapy were associated with a constant lower risk of mortality (Table). Interestingly, the effects of ER-PR- and radiotherapy on mortalities changed over time, which did not comply with the constant-HR assumption. At the time of diagnosis and treatment, the ER-PR- patients were more likely to die compared to the patients with ER+PR+; however, the likelihood of death decreased over time, and after around 66 months, having ER-PR- IBC became less likely to die compared to the patients with ER+PR+ (Figure). The patients with radiotherapy were less likely to die compared to the patients without it at the beginning, then this protective effect diminished

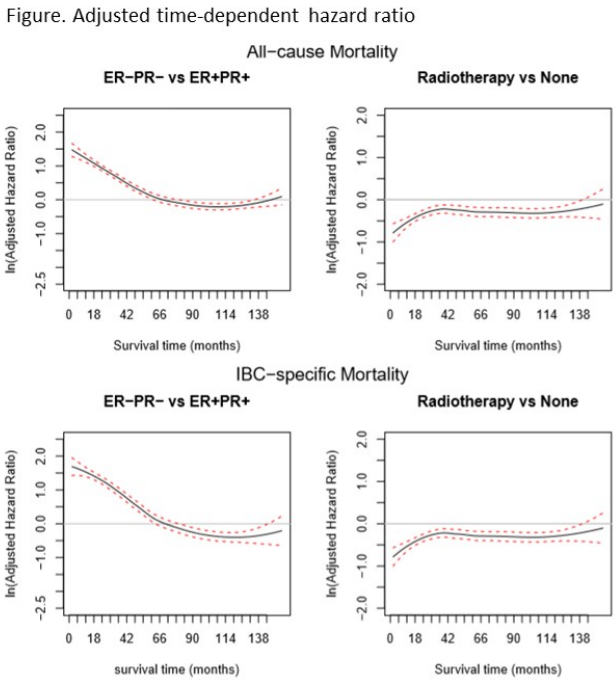
over time, and after around 36 months, it stayed plateau throughout the rest of the study period (Figure). Surprisingly, there was no difference in all-cause mortality among different histologic types. In the IBC-specific mortality analysis, the patients with mixed ductal and lobular carcinoma had a slightly higher risk compared to the patients with IDC (Table)

Table. Adjusted hazard ratios for risk of all-cause mortality and IBC-specific mortality (N=34013)

Potential prognostic factors (N, %)	All-cause mortality Adjusted hazard ratio (95% CI)	IBC-specific mortality Adjusted hazard ratio (95% CI)
Menopause (age ≥ 50 years)		
No (9234, 27%)	Reference	Reference
Yes (24779, 73%)	2.12 (2.01, 2.23)	1.22 (1.14, 1.29)
Race		
White (27951, 82%)	Reference	Reference
Black (3418, 10%)	1.30 (1.23, 1.38)	1.39 (1.29, 1.50)
Other (2644, 8%)	0.70 (0.64, 0.76)	0.73 (0.65, 0.82)
Histologic type		
IDC (25069, 73.7)	Reference	Reference
IDCMO (1046, 3.1%)	1.06 (0.94, 1.17)	0.98 (0.82, 1.17)
ILC (2001, 5.9%)	1.01 (0.93, 1.09)	1.05 (0.93, 1.18)
ILCMO (153, 0.4%)	0.79 (0.58, 1.07)	1.06 (0.69, 1.63)
MDLC (2981, 8.8%)	0.99 (0.93, 1.07)	1.12 (1.02, 1.24)
Other (2763, 8.1)	1.06 (0.99, 1.13)	0.93 (0.84, 1.03)
Histologic grade		
1 or 2 (20963, 62%)	Reference	Reference
3 (13050, 38%)	1.26 (1.21, 1.32)	1.57 (1.48, 1.68)
pT		
pT1 or pT2 (30943, 91%)	Reference	Reference
pT3 or pT4 (3070, 9%)	1.91 (1.80, 2.02)	2.09 (1.95, 2.25)
pN		
0 (21975, 65%)	Reference	Reference
1 (8246, 24%)	1.48 (1.41, 1.55)	2.10 (1.96, 2.25)
2 (2460, 7%)	2.44 (2.28, 2.61)	3.77 (3.46, 4.11)
3 (1332, 4%)	3.28 (3.03, 3.55)	5.12 (4.65, 5.63)
pM		
No (33099, 97%)	Reference	Reference
Yes (914, 3%)	3.02 (2.78, 3.29)	4.03 (3.66, 4.44)
Hormone Status		
ER+PR+ (21669, 64%)	Reference	Reference
ER- PR- (7596, 22%)	Time-dependent	Time-dependent
ER+PR- (4300, 13%)	1.12 (1.06, 1.18)	1.34 (1.23, 1.45)
ER- PR+ (448, 1%)	0.94 (0.79, 1.12)	1.23 (0.99, 1.52)
Surgery		
No (626, 2%)	Reference	Reference
Yes (33387, 98%)	0.47 (0.43, 0.52)	0.43 (0.38, 0.48)
Chemotherapy		
No (18317, 54%)	Reference	
Yes (15696, 46%)	0.56 (0.54, 0.59)	0.92 (0.86, 0.98)
Radiotherapy		
No (15288, 45%)	Reference	Reference
Yes (18725, 55%)	Time-dependent	Time-dependent

IBC: invasive breast cancer
CI: confidence interval
IDC: invasive ductal carcinoma
ILC: invasive lobular carcinoma
MDLC: mixed IDC and ILC
IDCMO: IDC mixed with other types of carcinoma
ILCMO: ILC mixed with other types of carcinoma

Figure 1 - 205



Conclusions: Hormone status and radiotherapy are time-dependent prognostic factors for IBC, which needs to be adjusted in modelling IBC survival. More studies on the mechanism of time-varying predictors are needed.

206 FGF13 Suppresses Tumor Proliferation and Invasion by Regulating EMT in Breast Cancer

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Disclosures: Yueping Liu: None; Hanxu Jiang: None

Background: Breast cancer is the most common malignancy in women, and its incidence is increasing worldwide. There is currently no effective therapy to control the recurrence and metastasis of breast cancer. The fibroblast growth factor 13 (FGF13) was shown to be up-regulated in various cancers and associated with the accumulation of reactive oxygen species and apoptosis. However, the role of FGF13 in breast cancer (BC) progression and metastasis remains incompletely understood. In the present study, we investigated the effects of FGF13 on BC metastasis and proliferation, as well as the underlying molecular mechanisms.

Design: 13 ductal invasive carcinoma tissues and 8 ductal carcinoma in situ tissues were collected between January 2019 and August 2019 in the Fourth Hospital of Hebei Medical University .The expression of FGF13 mRNA levels was compared by real-time quantitative polymerase chain reaction（RT-qPCR）.Then we analyzed the role of FGF13 in breast cancers based on database. Overexpression of FGF13 in two BC cell lines, MDA-MB-231 and MCF-7, MTS assays and clone formation assays were used to detect breast proliferation, wound healing Assay and transwell test were used for observing breast cancer migration. Next, we used RT-qPCR and Western blot to detect the expression of EMT (epithelial-mesenchymal transition) markers mRNA and protein levels.

Results: Bioinformatics database analysis indicated that the high expression of FGF13 was closely associated with good survival in BC. Clinical results showed that FGF13 expression was higher in ductal carcinoma in situ than that in invasive carcinoma (p<0.05). Then we found that FGF13 inhibited the proliferation of BC cells in MTS and clone formation experiments. (p<0.05). Transwell assay demonstrated that FGF13 diminished cell migration of BC cells(p<0.05). Additionally, the overexpression of FGF13 in BC cell line resulted in the induction of increase in E-cadherin(CDH1).

Figure 1 - 206

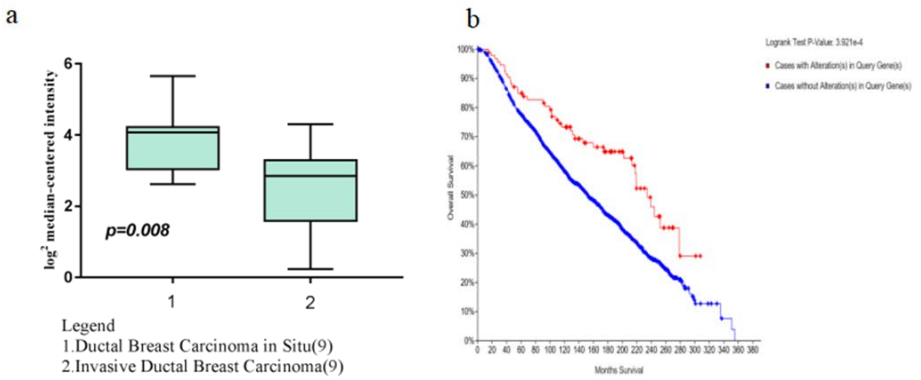

Fig. 1 Bioinformatics analysis of FGF13 expression in human breast cancer.a.Box plots of FGF13 gene expression between ductal breast carcinoma in situ and invasive ductul breast carcinoma(Oncomine database). b.Kaplan–Meier survival analysis illustrating the correlation between higher FGF13 expression and Increased overall survival in breast cancer patients from the cBioportal database.

Figure 2 - 206

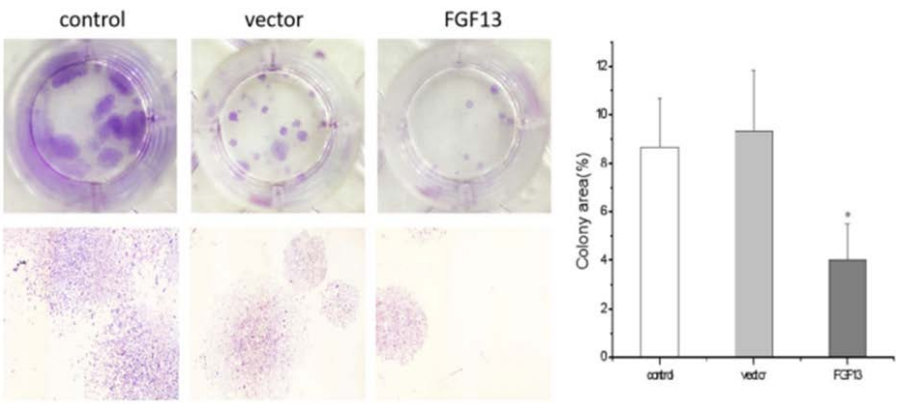

Fig. 2.FGF13 inhibits proliferation and colony formation in MDA-MB-231 cells. Colony formation assays performed on MDA-MB-231 cells transfected with FGF13 or control vector. *$P < 0.05$.

Conclusions: Taken together, our results suggest that FGF13 acts as a tumor suppressor that negatively regulates BC cell proliferation and migration, and the effect on breast cancer migration may be linked to the EMT process.

207 Mammary Desmoid-Type Fibromatosis: A 15-Year Single Institution Experience

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Disclosures: Christina Luffman: None; Gabrielle Baker: None; Laura Collins: None; James Connolly: None; Ashley Ward: None; Liza Quintana: None

Background: Desmoid-type fibromatosis (DTF) is a rare clonal proliferation of fibroblasts/myofibroblasts. DTF are locally aggressive and have the potential for local recurrence (LR) but do not metastasize. Mammary DTF (MDTF) is similar to DTF elsewhere and represents approximately 0.2% of all breast tumors. The etiology is unknown; however, there is an association with prior trauma and genetic factors

(e.g. Gardner-type familial adenomatous polyposis [GTFAP]). Treatment paradigms are shifting toward observation rather than excision for DTF, as such we sought to review our institutional experience with this rare neoplasm.

Design: MDTF diagnosed between 2004-2019 were retrospectively identified. Clinical and pathology data were reviewed.

Results: 31 patients with MDTF were identified, 28 of whom underwent surgical excision with a mean follow-up of 3.5 years (0 to 10 years); 3 patients were lost to follow-up. 29 (94%) were women. The median age was 41 years (range 19-84). 18 (58%) presented with a palpable mass. Average size on imaging was 1.9 cm (range 0.5-10.5). Prior history of potential trauma was noted for 10 (32%) patients, including 9 with prior biopsies or surgery. 2 patients had additional DTF: 1 with an abdominal wall DTF and 1 (with GTFAP) had multiple DTF and Gardner fibromas. B-catenin IHC was performed on all cases; 26 (84%) had positive nuclear staining. Of 19 patients with positive surgical margins, 8 were re-excised. At last follow-up, 30 patients had not experienced a LR. None of 10 patients with positive margins experienced LR (1 was lost to follow-up). One patient with negative margins recurred 13 months after surgery; 15 months after excision for the LR the patient remained disease free. The patient with GTFAP was treated with Sundilac for a subsequent abdominal DTF. 2 patients declined excision and had no progression 1 and 2 years later.

Conclusions: The treatment for DTF is evolving with contemporary literature suggesting active surveillance, however, data are limited. In our cohort, the 2 patients who declined excision experienced no change and a slight decrease in tumor size at 1 and 2 years, respectively. Ten cases with final positive margins did not experience a LR. LR occurred in 1 patient with negative resection margins of the initial MDTF. Our data support a conservative approach for the management of MDTF. Further investigation of factors related to risk for LR/progression in MDTF, including chromosomal alterations and *CTNNB1*-mutation is needed.

208 Evaluation of Subareolar Tissue Biopsies in Predicting Occult Nipple Involvement in Nipple-Sparing Mastectomies

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Disclosures: Lucy Ma: None; Paula Ginter: None

Background: Nipple-sparing mastectomy (NSM) is an increasingly utilized oncologic surgical procedure; however, there is risk of residual malignancy lingering in the preserved nipple areolar complex (NAC). To minimize this risk, routine subareolar tissue sampling for pathologic evaluation is performed. Malignant diagnoses typically warrant excision of the nipple/NAC. The rate of residual malignancy in nipple/NAC excisions following a positive subareolar biopsy has not been widely studied. We sought to determine the rate of residual carcinoma in nipple/NAC excisions following a positive subareolar biopsy and assess the value of intraoperative frozen section (IOF) of subareolar biopsies.

Design: We identified 1026 consecutive NSMs with separately submitted subareolar biopsies between 12/2013 and 05/2019. Biopsies containing invasive carcinoma and/or ductal carcinoma in situ (DCIS) were considered positive. Concordance rates between subareolar biopsies and subsequent excisions were examined as well as IOF and permanent diagnoses. Clinicopathologic data were reviewed and statistical analyses were performed using chi-square test.

Results: Of the 1026 NSMs, 575 (56 %) were therapeutic and 451 (44 %) were prophylactic [Figure 1]. Only 5% (51/1026) of subareolar biopsies were positive [Table 1]. Of the positive subareolar biopsies, 78% (40/51) underwent subsequent excision with 50% (20/40) showing residual malignancy. Positive biopsies were significantly associated with multifocal/multicentric disease when compared to negative biopsies (p=0.002). There was no statistically significant difference in likelihood of biopsy positivity between tumors ≤2 cm vs. >2 cm from the nipple (p>0.05). IOF was performed in 273 cases (27%), of which 4 (1.5%) demonstrated a discrepancy between frozen and permanent diagnoses. In 3 discrepant cases, malignancy was only present on permanent section. In 1 case, the IOF was interpreted as malignant; however, on the permanent slide, the focus was shown to be benign (i.e. true false positive).

Table 1. Characteristics of Positive Subareolar Biopsies in Nipple Sparing Mastectomies.

Positive subareolar biopsy, N=51	N (%)
Subareolar biopsy diagnosis DCIS Invasive ductal carcinoma Invasive lobular carcinoma	33 (64.7%) 11 (21.6%) 7 (13.7%)
Multifocal/Multicentric Yes No	40 (78.4%) 11 (21.6%)
Distance (cm) to the nipple, known 0 – 2 >2 – 4 >4 – 6 >6	N=37 16 (43.2%) 8 (21.6%) 6 (16.2%) 7 (18.9%)
Frozen section Yes No	35 (68.6%) 16 (31.4%)
Frozen section diagnosis DCIS Invasive carcinoma Atypical Highly suspicious for carcinoma Benign	N=35 12 (34.3%) 7 (20%) 3 (8.6%) 2 (5.7%) 11 (34.4%)*
Nipple/NAC excision diagnosis DCIS Invasive ductal carcinoma Invasive lobular carcinoma LCIS Benign	N=40 13 (32.5%) 6 (15%) 1 (2.5%) 4 (10%) 16 (40%)
*malignancy was present on permanent section in 3 cases and in 8 cases additional subareolar biopsy specimens submitted for permanent section were malignant	

Figure 1 - 208

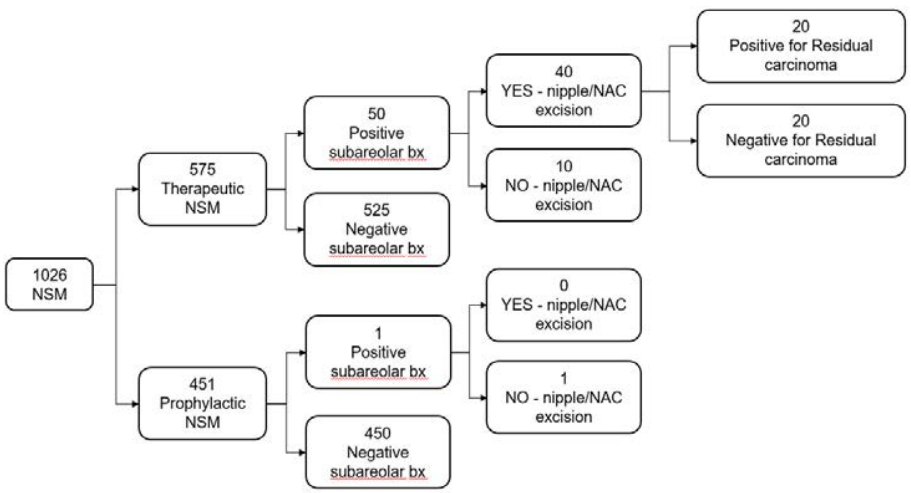

Figure 1. Distribution of subareolar biopsy (bx) and subsequent nipple/nipple areolar complex (NAC) excision results in 1026 nipple-sparing mastectomies (NSM).

Conclusions: While the rate of positive subareolar biopsies in NSM is low, positive subareolar biopsy predicts the presence of residual occult carcinoma in half of nipple/NAC excisions. Positive subareolar biopsies were more frequently seen in patients with multifocal/multicentric disease. Additionally, IOF is accurate and reliable for subareolar biopsy diagnosis.

209 Patients with Benign Papilloma Diagnosed on Core Biopsies and Concordant Pathology-Radiology Findings can be Followed: A Prospective Study

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Disclosures: Zhongliang Ma: None; Cletus Arciero: None; Toncred Marya Styblo: None; Haibo Wang: None; Michael Cohen: None; Xiaoxian Li: None

Background: Although there is a national trend to spare patients from surgery when a benign papilloma is diagnosed on image-guided core biopsy, the data remain controversial.

Design: Based on the results of published retrospective studies and our own experiences, we prospectively reviewed the clinical history, imaging, and pathology of all contemporaneous papilloma cases in a multispecialty conference attended by breast pathologists, imagers, and surgeons to develop a consensus management recommendation to either excise the papilloma or to follow with imaging and clinical evaluation at 6 month intervals for a minimum of two years.

Results: A total of 150 core biopsy-diagnosed papilloma cases were prospectively reviewed. Of the 150 cases, 148 were determined to have concordant radiologic-pathologic features. 118 of these 148 were benign papillomas of which 39 were excised with no upgrades to invasive carcinoma or DCIS. Reasons for surgical excision included patient preference or symptomatic relief (ex: nipple discharge). Of the remaining 79 benign papillomas which were not excised, there were no cases of malignancy during follow-up (39-1279 days, mean 531 days). Two patients were lost to follow-up within one month of the core biopsy. Eleven cases revealed atypical ductal hyperplasia (ADH) or atypical lobular hyperplasia (ALH) adjacent to, but separate from, a benign papilloma. Of these, 6 were excised with 2 upgrades to invasive carcinoma or DCIS. In both upgraded cases, ADH bordering on DCIS was noted on the core biopsy; the remaining 5 patients did not have evidence of malignancy during follow-up (181-325 days except for one patient was lost to follow-up within 1 month). The remaining 19 of the 150 core biopsies were 17 atypical papillomas (i.e. papillomas containing ADH) and 2 papilloma involved by ALH. Of these, 15 atypical papilloma were surgically excised with 4 upgraded to invasive carcinoma or DCIS; the remaining 4 patients (2 atypical papilloma and 2 papilloma involved by ALH) did not show evidence of malignancy during follow-up (105-993 days). Finally, 2 cases were determined rad-path discordant with no upgrade to malignancy at subsequent surgical excision.

Conclusions: Our data confirm that rad-path concordant benign papillomas diagnosed on image-guided core biopsy can be spared from surgery. It also supports the value of a formal multispecialty review of all core biopsy-diagnosed papilloma cases to derive a prospective consensus management plan for each case.

210 Benign High-Risk Breast Lesions can Avoid Unnecessary Surgery: A Prospective Study

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Disclosures: Zhongliang Ma: None; Cletus Arciero: None; Toncred Marya Styblo: None; Haibo Wang: None; Michael Cohen: None; Xiaoxian Li: None

Background: Managing high-risk breast lesions following their diagnosis on imaging-guided core biopsy remains controversial.

Design: Based on more recent publications and our own retrospective data, we developed a high-risk breast conference attended by breast pathologists, breast surgeons, and breast imagers to prospectively review each case to arrive at a consensus recommendation to either surgically excise or follow on imaging at 6 month intervals for a minimum of two years.

Results: Between 2015 and 2019, 128 high-risk lesions were discussed. Of these 128 cases, 120 had concordant pathology-radiology findings. The remaining 8 patients with discordant pathology-radiology findings underwent excision and 2 were upgraded to carcinoma. Of the 120 concordant cases, there were 45 with ADH (3 with concurrent LCIS and 1 with ALH), 13 with LCIS, 23 with ALH, 36 with RS, 2 with FEA and 1 with a mucocele-like lesion. Surgical excision was recommended for cases of ADH when there were >2 ADH foci or <90% of the calcifications were removed at core biopsy or there was severe nuclear atypia or punctate necrosis. Surgical excision of ALH and LCIS was recommended when there were >2 foci or calcifications were present within the ALH or LCIS. For cases of RS, excision was recommended when it was estimated less than half of the lesion was sampled. Based on these criteria, we recommended to excise 27 of the 45 ADH cases and of these, 9 were upgraded to carcinoma. Seven of the 13 with LCIS were recommended for surgery with one

upgrade to invasive lobular carcinoma. Three of 23 with ALH and 9 of 36 with RS were recommended for excision with none upgraded. An additional 4 patients with ADH, 1 with LCIS, 1 with ALH, and 4 with RS underwent voluntary surgery and none were upgraded. All other patients (14 with ADH, 5 LCIS, 4 ALH, 23 RS, 2FEA and 1 mucocele –like lesion) were followed with imaging every 6 months and none have had any evidence of malignancy (32-1389 days, mean 624 days).

Conclusions: This prospective study indicates that high risk lesions can be successfully triaged to surgery versus observation if rigorous radiologic-pathologic correlation is performed with establishment of pre-defined firm guidelines. High-risk case conference attended by breast pathologists, surgeons, and radiologists is an effective means of reaching consensus on the management of all high-risk lesions sampled in our institution.

211 Molecular Characterization of Microglandular Adenosis and Associated Invasive Carcinomas

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Disclosures: Sundis Mahmood: None; Liza Quintana: None; Jonathan Marotti: None; Joel Lefferts: None; Kristen Muller: None

Background: Although microglandular adenosis (MGA) of the breast is regarded as a benign lesion, a few studies investigating the genetic landscape of MGA and associated invasive carcinomas have provided evidence into its neoplastic potential. Identical somatic mutations, most frequently in *TP53,* and similar patterns of chromosomal alterations have been reported in MGA and associated invasive carcinomas. In addition, mutations in PI3K pathway-related genes (*PTEN, PIK3CA, and INPP4B*) and tyrosine kinase receptor signaling-related genes (*ERBB3* and *FGFR2*) have been implicated in *TP53* wild-type MGA. We report the clinicopathological and genomic features of four additional cases of MGA with associated invasive carcinoma.

Design: A pathology database search for MGA revealed four cases; three cases had associated invasive carcinoma. Areas of MGA and invasive carcinoma from each case were dissected and subjected to next-generation sequencing (NGS) for somatic mutations on the 50-gene AmpliSeq Cancer Hotspot Panel v2 on the Ion Torrent PGM (318 chip) and single nucleotide polymorphism-based chromosomal microarray analysis (CMA) using the Oncoscan FFPE Assay Kit (Affymetrix, ThermoFisher) to assess the tissue for copy number changes and copy neutral loss of heterozygosity across the entire genome.

Results: Results are presented in table 1. Two cases (1 and 2) of MGA were intimately associated with high grade, triple-negative invasive carcinomas with matrix production. CMA of these tumors revealed similar profiles with high levels of genomic instability across the entire genome in the MGA and invasive carcinoma components. NGS revealed co-occurring somatic mutations in *TP53* and PI3K pathway-related genes in the invasive carcinoma in case 1. Case 2 had identical *TP53* stopgain mutations in the MGA and invasive components. In contrast, the low-grade, triple-negative invasive ductal carcinoma and associated MGA in case 3 were significantly less complex and more genomically stable. Case 4 contained a microscopic focus of MGA discovered incidentally in reduction tissue removed from a patient with an ER-positive invasive lobular carcinoma in a different quadrant; the MGA showed a normal CMA profile and a single somatic mutation in *PIK3CA*.

Table 1. Genomic characterization of MGA and associated invasive carcinoma

	CMA		NGS	
	MGA	**Invasive**	**MGA**	**Invasive**
Case 1 – TN high-grade invasive carcinoma with chondromyxoid matrix production	High level of genomic instability with numerous low-level gains and losses across the entire genome	High level of genomic instability with numerous low-level gains and losses across the entire genome	Failed QC	*FBXW7* *PTEN* *TP53*
Case 2 – TN high-grade invasive carcinoma with metaplastic features/matrix production	High level of genomic instability with numerous low-level gains and losses across the entire genome	High level of genomic instability with numerous low-level gains and losses across the entire genome	*TP53**	*TP53** *FGFR2*
Case 3 – TN low-grade invasive ductal carcinoma	Normal	Low-level gain chr.2 Copy neutral LOH chr.10	Failed QC	Failed QC
Case 4 – MGA discovered incidentally in reduction tissue; ER+ invasive lobular carcinoma in different quadrant	Normal	N/A	*PIK3CA*	N/A

TN = triple-negative; *Identical *TP53* stopgain c.1024C>T, p.R342 mutation

Conclusions: This study demonstartes the genetic heterogeneity of MGA. MGA associated with high-grade tumors has highly complex genomic alterations with frequent *TP53* mutations, while MGA associated with low-grade tumors is more genomically stable with few chromosomal aberrations.

212 MET Gene Amplification in Breast Cancer

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Disclosures: Leonel Maldonado: None; Ferrando Lorenzo: None; Mahsa Vahdatinia: None; Dara Ross: None; Ahmet Zehir: *Speaker*, Illumina; Hannah Wen: None; Edi Brogi: None; Britta Weigelt: None; Sarat Chandarlapaty: *Consultant*, BMS; *Consultant*, Novartis; *Consultant*, Lilly; *Consultant*, Sermonix; *Consultant*, Revolutions Medicine; Pedram Razavi: *Consultant*, Novartis; *Grant or Research Support*, Grail Inc; Jorge Reis-Filho: None; Fresia Pareja: None

Background: *MET* encodes for the receptor tyrosine kinase c-MET. *MET* gene amplification and exon 14 skipping mutations are found in a small subset of human cancers, and have been shown to constitute useful therapeutic targets in non-small cell lung cancer and renal cell carcinoma. Here we sought to determine the frequency of potentially targetable *MET* genetic alterations in breast cancers (BCs) and characterize the clinicopathologic features of BCs with *MET* alterations.

Design: We retrospectively queried 5,575 BCs previously analyzed by clinical MSK-IMPACT targeted sequencing and investigated the presence of *MET* gene amplification and exon 14 skipping mutations. A central histopathologic review of BCs found to harbor the aforementioned genetic alterations was conducted. Clinical characteristics and estrogen receptor (ER)/HER2 status determined as per the ASCO/CAP guidelines were retrieved from the clinical records.

Results: Of the BCs interrogated, 0.2% (n=10) harbored *MET* gene amplification including 3 primary BCs (p-BCs) and 7 metastatic BCs (m-BCs). No BCs harboring *MET* exon 14 skipping mutations were identified. The median age of the patients was 53 years (range, 38-72). The p-BCs included two invasive ductal carcinomas of no special type (IDC-NSTs) and 1 metaplastic breast cancer with predominant chondroid component, all of histologic grade 3. Two p-BCs were ER-negative/HER2-negative and one was ER-positive/HER2-negative, and all of them harbored *TP53* somatic mutations. The *MET*-amplified m-BCs included 5 IDC-NSTs (67%), one metaplastic breast cancer with a predominant chondroid component and one pleomorphic lobular carcinoma. All m-BCs were poorly differentiated, 3/7 were ER-negative/HER2-negative (43%), 2/7 were ER-positive/HER2-negative (29%), 1/7 was ER-positive/HER2-positive and 1/7 was ER-negative/HER2-positive. Metastatic sites included pleura (2/7), liver (2/7), lymph node (1/7), skin (1/7) and bone (1/7). Consistent with the findings in *MET*-amplified p-BCs, the only gene recurrently mutated in *MET*-amplified m-BCs was *TP53.*

Conclusions: *MET* gene amplification is remarkably rare in BCs. *MET*-amplified BC is phenotypically heterogeneous, but appears to be enriched in high-grade ER-negative/HER2-negative phenotype harboring *TP53* mutations.

213 Duct vs. Lobule: Is Location a Useful Feature in Breast Cores with Low Grade Ductal Proliferations?

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Disclosures: Kiran Manjee: None; Megan Sullivan: None

Background: Atypical ductal hyperplasia (ADH) and grade 1 ductal carcinoma in-situ (DCIS) share many architectural and cytologic features and differentiating the two can be challenging on core biopsy (CB). When either is diagnosed on CB, the next step is surgical excision (EX). However, patients with DCIS often undergo radiotherapy and hormonal therapy, regardless of the excision findings. This difference in treatment makes interpretation of the CB especially important. In this study we examined whether the location of atypia could be a useful diagnostic feature.

Design: The database was searched for CB diagnosis (DX) of ADH or DCIS. Patients with ipsilateral breast cancer, MRI or US guided CB and unavailable EX were excluded. A pathologist blind to the EX DX reviewed slides for 138 CB. 16 cases were excluded for not meeting criteria for ADH/DCIS or unavailable slides. 122 patients' CB were classified into 3 categories based on the original and reviewed DX. Category 1 is concordant ADH (both DX ADH). Three types of CB fall into category 2: CB DX as "ADH bordering DCIS", CB with different original and review DX and patients with 2 ADH CB in one breast. Category 3 is concordant DCIS (both DX DCIS). Features evaluated included location [ductal (D), lobular (L) or mixed (DL)], # of foci and architectural patterns. The results were correlated with the EX; DCIS or invasion is considered an upgrade.

Results: While the average # of foci was similar, the L predominant pattern was seen most often in category 1 while all category 3 CB were D predominant (Table 1). Regardless of category, 8/56 L predominant CB were upgraded (14%) while 9/30 D predominant CB were upgraded (30%). The majority of upgrades had a cribriform component (22/27; 81%). Of 8 L predominant upgrades, 7 (87%) had a cribriform pattern; only one was micropapillary. Category 2 encompassed the challenging cases. Of 15 borderline CBs, 1 upgrade was L predominant. 10 CB were discordant (original and reviewed DX differed) and no upgrades were L predominant.

Category	N	Excision Upgrades	D	DL	L	Average # foci
1 (ADH-ADH)	84	17 (20%)	24 (29%)	13 (15%)	47 (56%)	2.1
2-All cases	29	10 (34%)	6 (21%)	14 (48%)	9 (31%)	2.8
2-Borderline	15	5 (33%)	1 (7%)	9 (60%)	5 (33%)	3.4
2-Discordant	10	3 (30%)	5 (50%)	1 (10%)	4 (40%)	3.0
2-Two site ADH	4	2 (50%)	0	4 (100%)	0	1.4
3 (DCIS-DCIS)	9	0	9 (100%)	0	0	2.3

Conclusions: ADH vs. DCIS is a common dilemma and location may be a helpful feature. CB with a consensus DX of ADH were more likely to be L predominant and had a lower upgrade rate. The upgrade rate for D predominant ADH was twice than that of L predominant ADH. L predominant cases with cribriforming were more likely to be upgraded. In discordant CB, none of the L predominant cases were upgraded. Our data suggests that CB with L predominant atypia are best classified as ADH.

214 PDL1 Expression and Tumour Infiltrating Lymphocytes in Triple-Negative Breast Cancer and its Correlation with Clinicopathological Features and Response to Chemotherapy

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Disclosures: Sandeep Mathur: None; Anjana Johnson: None; Deo SVS: None; Ajay Gogia: None; Shruti Kahol: None; Deepali Jain: None; Venkateswaran Iyer: None

Background: Approximately, 25% of the female cancer cases in India are breast cancer (BC). Triple-negative breast cancer (TNBC) is the most aggressive form. The evaluation of tumor-infiltrating lymphocytes (TILs) in BC is gaining importance. High levels of Tregs in the tumor microenvironment are associated with poor prognosis in many cancers. Programmed death-ligand 1 (PD-L1) is a protein that inhibits the immune response through interaction with receptor PD-1 expressed on T cells. Thes aims of this study were to assess the influence of TILs, Tregs and PDL1 expression in TNBCs and correlate these with known prognostic markers and survival.

Design: 100 cases of TNBC were used. Follow up data of these patients with regards to distant metastasis, recurrence or death were retrieved from records to obtain survival & outcome parameters. H&E slides were evaluated for stromal TILs. Immunohistochemistry for PDL1 & Foxp3 were performed in all cases. Correlations of Clinico-pathological parameters were done with TILs, PDL-1, and FOXP3 expression. Survival data were also correlated with TILs, PDL-1 and FOXP3. Correlations were done with the response to chemotherapy. Kaplan-Meier time to event survival analysis was carried out to see the effect of subtypes on survival and recurrence of death among patients.

Results: 93% were invasive ductal carcinomas NST. 48% of cases were early BC, 24% were locally advanced BC and 28% were metastatic BC. 36% had received neoadjuvant chemotherapy the path-CR rate of 22.22%. There was a significant correlation between the level of TILs and tumor size, lymph node metastasis, lymphovascular invasion, anatomic staging, and prognostic staging. High level of TILs (>40%) were associated with improved overall survival (OS). There was a significant correction between FOXP3 on TILs and anatomic and prognostic staging. Higher FOXP3 level was associated with reduced overall survival. PDL-1 positivity on tumor cell was associated with higher anatomic and prognostic staging. There was a significant correlation between PDL-1 on lymphocytes and anatomic staging, prognostic staging, tumor size & OS.

Figure 1 - 214

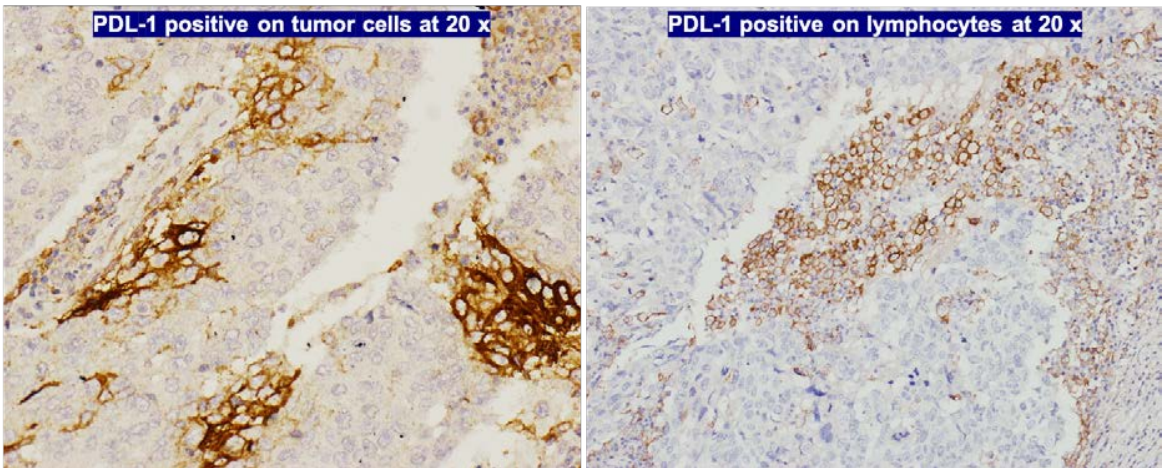
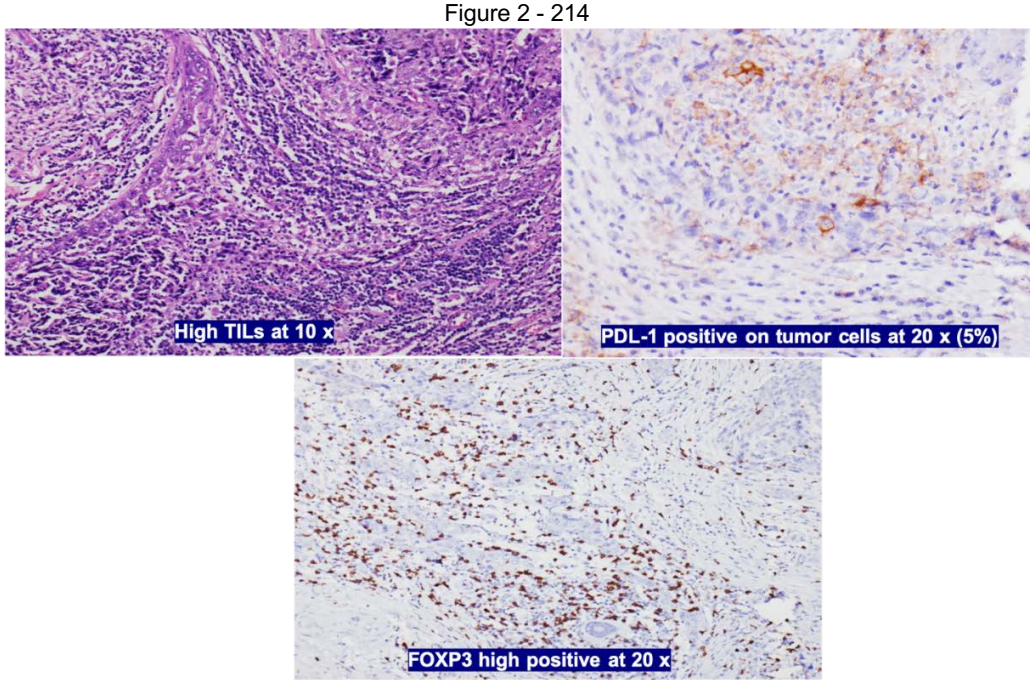


Figure 2 - 214



Conclusions: A higher level of TILs was associated with lower stage, lower tumor size, absence of lymph node metastases, decreased distant metastasis & improved OS. Higher FOXP3 T regs were also associated with higher anatomic and prognostic staging. PDL-1 expression on both tumor cells and lymphocytes was associated with higher stage, decreased OS and poor clinical outcome.

215 Whole Exome Sequencing Analysis of Local/Regional and Distant Metastatic Breast Carcinoma

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Disclosures: Patrick McIntire: None; Paula Ginter: None; Dayle Lapolla: None; Kenneth Eng: None; Shaham Beg: None; Alexandros Sigaras: None; Jyothi Manohar: None; Noah Greco: None; Tuo Zhang: None; Rohan Bareja: None; Andrea Sboner: None; Olivier Elemento: None; Eleni Andreopoulou: None; Juan Miguel Mosquera: None

Background: Patients with metastatic breast carcinoma (MBC) who fail first line therapies may have limited options. Molecular sequencing can provide information to guide salvage therapies. Our study aim was to describe the whole-exome sequencing (WES) profile of MBC from patients enrolled in our Precision Oncology program, with an emphasis in alterations on pathways that may have therapeutic ramifications.

Design: WES was performed on tumor/matched germline DNA pairs from patients with MBC. Our WES test allows for assessment of >21,000 genes through the development and implementation of established computational pipelines for simultaneous detection of somatic point/indel mutations and copy-number alterations (CNAs).

Results: The cohort was 27 cases of MBC from 24 patients including 12 cases of regional or axillary lymph node metastases and 15 cases of distant metastases (liver, bone, brain, pleura, skin, soft tissue). The biomarker tumor profile by IHC was 18 (67%) ER+, 17 (63%) PR+, 5 (19%) HER2+ and 4 (15%) were triple negative. 25 (93%) were ductal, 1 (4%) lobular and 1 (4%) was metaplastic carcinoma. 12 patients (50%) had somatic alterations in 10 clinically relevant genes including *BRCA1, FGFR1, FGFR2, PIK3CA, FGFR2, ERBB2, KIT, PTEN, AURKA, SMO* and *AKT1*. (Fig. 1). 2 patients were considered extreme responders.

The most frequent alterations were *TP53* (9/24, 39%) and *PIK3CA* (4/24, 17%). Other frequent alterations involved *ERBB2* (5/22, 3 amplifications, 2 activating mutations). 2 (8%) cases had high tumor mutational burden (>12 mutations per Mb). Based on inclusion criteria, 10 (42%) patients would be eligible for enrollment in molecularly driven clinical trials. Further analysis showed that 9 (38%) MBC had alterations in the PI3K-AKT-mTOR pathway (17 genes), 11 (46%) in the cell cycle control (34 genes), 7 (29%) in the Notch signaling pathway (55 genes), 2 (8%) in the AR signaling pathway (10 genes), 1 (4%) in folate transport (5 genes), and 4 (17%) in DNA damage response (12 genes). 3 of the latter were germline variants (two *BRCA2* and one *ATM*) (Fig. 2). Overall, 19 (79%) patients had molecular

profiles with potential therapeutic ramifications, including tumor susceptibility to PARP inhibitors or immunotherapy. No cases with microsatellite instability (MSI) were detected by MSI Sensor.

Figure 1 - 215

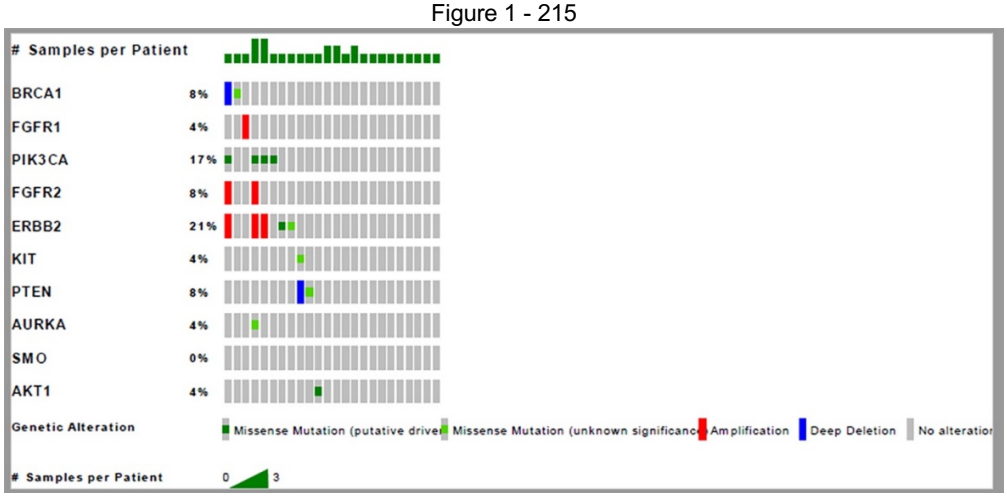
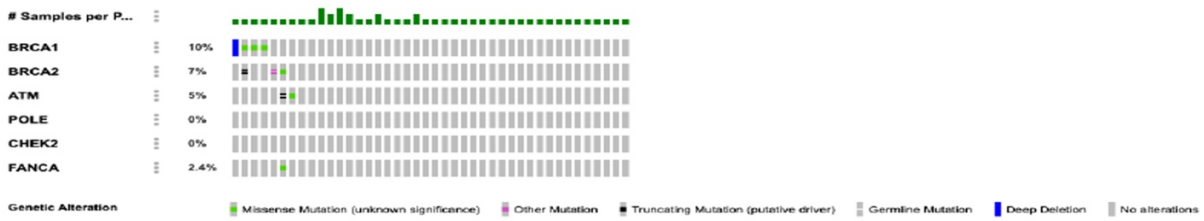


Figure 2 - 215



Conclusions: While targeted panels are paramount, routine WES including TMB and MSI scores, and somatic and germline analysis, may be informative on pathway alterations that could provide clinical benefit.

216 Androgen Receptor Expression in Patients with Triple Negative Breast Cancer Diagnosed at the Colombian National Cancer Institute

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Disclosures: Mario Melo-Uribe: None; Silvia Serrano-Gomez: None; Maria Abaunza: None

Background: Triple negative breast cancer (TNBC) is a subtype with an aggressive behavior and with no available molecular targets. It occurs most often in pre-menopausal African American and Hispanic/Latino women. In Colombia its prevalence has been reported in 20.6%. Androgen Receptor (AR) belongs to the steroid nuclear receptor family and has been recently considered a potential biomarker for breast cancer. Considering the high prevalence of TNBC in Colombian women and the lack of knowledge of AR expression in our patients, our aim was to determine the frequency of AR expression and its association with clinic-pathological variables in women diagnosed with TNBC at the Colombian National Cancer Institute (INC).

Design: This study included 149 women diagnosed with TNBC between 2011 and 2014 at the INC. Clinical and pathological data were extracted from medical records and pathology reports. Information on hormone receptor status (ER and PR), Ki67 expression and HER2 was obtained from previous pathology reports, if available. AR expression was considered positive when it exceeded 1% of nuclear staining in tumor cells.

Results: Androgen receptor (AR) expression was detected in 41.6% of the samples using a 1% cutoff. We explored differences in clinic-pathological variables according to the AR expression (Table 1). We found statistically significant differences in the mean age at diagnosis and histology. Patients that expressed AR between 1% and 50% of the receptor were older than patients negative for AR expression (60.78 yo vs. 53.78 yo, p = 0.043). A higher number of apocrine carcinomas were found in the group with the highest expression of AR compared to the group negative for the expression of the receptor (7.7% vs. 2.3%, p = 0.019). We did not find statistically significant differences in Ki67 expression, tumor size, metastasis at diagnosis, nodal stage, surgery, type of surgery, radiotherapy, recurrences and

death. The median of overall survival was 2.45 years (Figure 1). We did not find statistically significant differences in overall survival according to AR expression (Figure 2).

	Androgen receptor expression			
	< 1% (N=87)	**1% - 50% (N=23)**	**≥ 50% (N=39)**	***p***
Mean age at diagnosis	53.78 (12.49)	60.78 (13.30)	53.92 (10.32)	0.043
Ki67 expression	62.36 (28.06)	56.30 (36.25)	66.82 (27.45)	0.399
Histology				
Apocrine carcinoma	2 (2.3)	0 (0.0)	3 (7.7)	0.019
Invasive ductal carcinoma	84 (96.6)	21 (91.3)	35 (89.7)	
Metaplasic carcinoma	0 (0.0)	2 (8.7)	0 (0.0)	
Papillary carcinoma	1 (1.1)	0 (0.0)	1 (2.6)	
Tumor size				
T1-T2	21 (24.1)	4 (17.4)	11 (28.2)	0.732
T3-T4	60 (69.0)	16 (69.6)	26 (66.7)	
Unknown	6 (6.9)	3 (13.0)	2 (5.1)	
Metastasis at diagnosis				
M0	66 (75.9)	15 (65.2)	31 (79.5)	0.633
M1	4 (4.6)	2 (8.7)	3 (7.7)	
Unknown	17 (19.5)	6 (26.1)	5 (12.8)	
Nodal Stage				
N0	11 (12.6)	4 (17.4)	8 (20.5)	0.338
N1	37 (42.5)	7 (30.4)	17 (43.6)	
N2-3	33 (37.9)	8 (34.8)	12 (30.8)	
NX	0 (0.0)	1 (4.3)	0 (0.0)	
Unknown	6 (6.9)	3 (13.0)	2 (5.1)	
Surgery				
Yes	71 (81.6)	18 (78.3)	34 (87.2)	0.629
No	16 (18.4)	5 (21.7)	5 (12.8)	
Type of surgery				
Cuadrantectomía	13 (18.3)	5 (27.8)	8 (23.5)	0.924
Mastectomy	13 (18.3)	2 (11.1)	6 (17.6)	
Other	44 (62.0)	11 (61.1)	20 (58.8)	
Unknown	1 (1.4)	0 (0.0)	0 (0.0)	
Radiotherapy				
Yes	60 (69.0)	15 (65.2)	29 (74.4)	0.725
No	27 (31.0)	8 (34.8)	10 (25.6)	
Recurrence				
Yes	35 (40.2)	9 (39.1)	18 (46.2)	0.650
No	51 (58.6)	13 (56.5)	21 (53.8)	
Unknown	1 (1.1)	1 (4.3)	0 (0.0)	
Death				
Yes	52 (59.8)	16 (69.6)	23 (59.0)	0.660
No	35 (40.2)	7 (30.4)	16 (41.0)	

Table 1. Clinical and pathologic characteristics of the patients according to AR expression

Figure 1 - 216

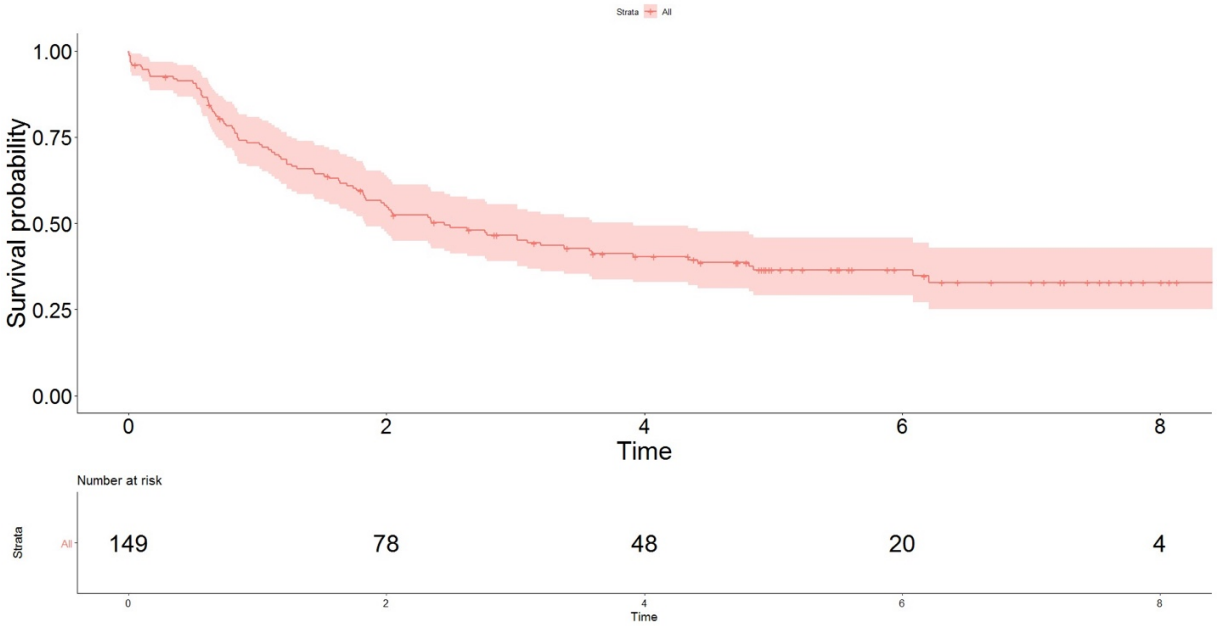
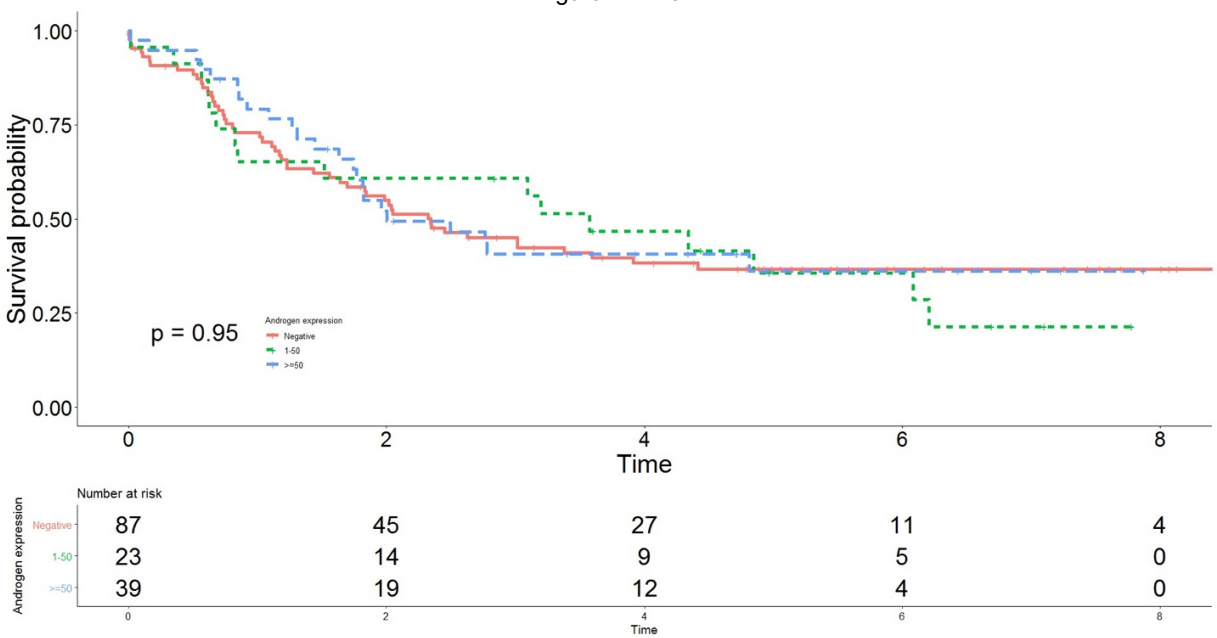


Figure 2 - 216



Conclusions: We found a high expression of AR in our samples from Colombian patients with TNBC (41.6%). We found statistically significant differences in AR expression by age at diagnosis and histology and no differences other clinic-pathological variables and survival. A larger sample size is needed to explore the prognosis value of AR expression in Colombian patients.

217 Discordance between Immunohistochemistry (IHC) and In Situ Hybridization (ISH) to Detect HER2 Overexpression/Gene Amplification in Breast Cancer (BC) in the Modern Age

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Disclosures: Raima Memon: None; Carlos Prieto-Granada: None; Shuko Harada: None; Thomas Winokur: None; Vishnu Reddy: None; Gene Siegal: None; Shi Wei: None

Background: HER2 gene amplification and/or protein overexpression reportedly occurs in 15-20% of BCs. Accurate detection of HER2 alteration is critical in predicting response to HER2-targeted therapy. Both IHC and ISH are FDA-approved methods for detecting HER2 status in BC, as HER2 protein overexpression is largely attributable to gene amplification. IHC is widely used and is the preferred method for screening while ISH is typically used as a reflex test on IHC-equivocal (2+) cases. Early studies have indicated that the discordant rates between IHC and ISH are up to 9%. In this study, we sought to determine the frequency of HER2 IHC–/ISH+ and IHC+/ISH- BCs in the modern age and to investigate the response to HER2-targeted therapy in these patients.

Design: All consecutive primary and metastatic BCs diagnosed between 01/2015-08/2019 with successful detection of HER2 by both IHC and Dual ISH at the authors' institution were included after IRB approval. Both IHC and ISH were interpreted by breast and molecular pathologists based on the 2013 ASCO/CAP HER2 testing guidelines.

Results: Of the 599 cases included in the study period, 75 (12.5%) were IHC-(2+), of which 11% (8) were ISH+. In the remaining cases, there was an overall 97.5% concordance rate between IHC and ISH, including 447 IHC-/ISH- and 64 IHC+/ISH+, respectively. Of the 13 (2.5%) BCs with discordant IHC/ISH, 5 were IHC-/ISH+ (including 1 metastatic BC) and 8 were IHC+/ISH-, respectively. Five patients (2 IHC-/ISH+ and 3 IHC+/ISH-) received HER2-targeted therapy at the authors' institution. Interestingly, one of these patients developed a HER2 IHC-/ISH+ distant metastasis from a primary BC with an IHC+/ISH+ phenotype 3 years after a pathologic complete response to neoadjuvant cytotoxic and HER2-targeted therapy. The remaining 4 patients were disease free.

Conclusions: A small subset of BCs show discordant HER2 IHC and ISH results, including IHC-/ISH+ and IHC+/ISH-. Thus, IHC scores of 3+ and 0/1+ should not be always regarded as positive and negative for HER2, respectively. A complementary test (ISH) is often needed. The potential biologic mechanisms driving these findings include nonfunctional amplifications or activating mutations which give rise to protein overexpression, while the phenotypic conversion from IHC+/ISH+ to IHC-/ISH+ may reflect tumor heterogeneity or a treatment effect. HER2 mutation analysis may provide further insights into the tumor biology of this highly diverse malignancy in the pursuit of precision medicine.

218 Intraductal Papilloma: To Excise or Not To Excise? A Review Of 286 Cases From a Single Institution

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Disclosures: Fatima Mir: None; Prih Rohra: None; Matthew Vega: None; Ritu Ghai: None

Background: Intraductal papillomas (IDPs) comprise 3-6% of breast biopsies diagnoses. IDPs can range from benign to atypical to harboring in-situ or invasive carcinoma. Management of benign IDP is controversial and different studies show varying results regarding surgical excision versus clinical observation. Our study aims to evaluate risk of malignancy in cases diagnosed as benign IDP on core biopsies, risk factors associated with increased risk of malignancy and determine if surgical excision is warranted.

Design: Our pathology database was searched for IDPs diagnosed on core biopsies (2010-june 2019). Total of 286 cases were identified, of which, 178 underwent excision. All patients were females (21-79 years). Clinical/radiologic data was reviewed.

Results: 202/286 cases were diagnosed benign IDPs on core biopsies. 109/202 underwent excision. 14/109(13%) were upgraded to high-risk lesions (atypical ductal/lobular hyperplasia). Imaging was available in 13/14; 8/13(61%) of these lesions were found in the central breast; 5/13(39%) peripheral. All of these lesions were detected on screening mammography except one which was detected as palpable mass. 7/109(6%) were associated with malignancy (ductal/lobular carcinoma in-situ and invasive carcinoma). In 4/7, there was a history of(h/o) contralateral(C/L) in-situ or invasive carcinoma; in 2/7, there was a strong clinical suspicion of malignancy; one case with ductal carcinoma in-situ was detected on screening mammogram. All these lesions were present either within or adjacent to the papilloma. 84/286 were diagnosed either as IDP with atypia or had coexisting in-situ/invasive carcinoma in the ipsilateral breast. 65/84 underwent excision at our institution. 56/65(86%) showed high-risk/malignant lesions on excision.

Conclusions: Our study shows that among lesions yielding a benign diagnosis of IDP, excision revealed high-risk lesions in 13% and malignancy in 6%. 61% of IDPs that were upgraded to high-risk lesions were located centrally. The likelihood of finding malignant lesion was significantly higher in women who presented with suspicious radiologic findings and/or had a h/o C/L breast cancer. We conclude that in cases of benign IDPs; personal history of C/L breast cancer, central location and highly suspicious imaging findings may be used as

criteria advocating surgical management. In patients diagnosed with peripherally located benign IDPs with no prior personal history of cancer, close clinical and radiological surveillance may be appropriate management.

219 Syringomatous Adenoma of Nipple (SAN), Low Grade Adenosquamous Carcinoma of Breast (LGASCA) and Microcystic Adnexal Carcinoma (MAC) of Skin: Molecular Analysis of these Rare Entities with Similar Histologic Features

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Disclosures: Mitul Modi: None; Ira Bleiweiss: None; Ronald Grenko: None; Shabnam Jaffer: None; Erik Toorens: None; Tapan Ganguly: None; Anupma Nayak: None

Background: SAN, LGASCA and MAC are extremely rare entities that share similar histologic and immunophenotypic features. They differ predominantly based on their location and biologic behavior. SAN is a benign tumor with superficial location and no propensity for metastasize, whereas LGASCA arises in the breast parenchyma, and is considered a variant of metaplastic carcinoma with potential to metastasize to axilla nodes and distantly. MAC is a locally aggressive cutaneous tumor and can metastasize. Given their rarity and histologic similarities they impose a diagnostic challenge to a pathologist and hence, we sought to uncover molecular underpinnings of these entities.

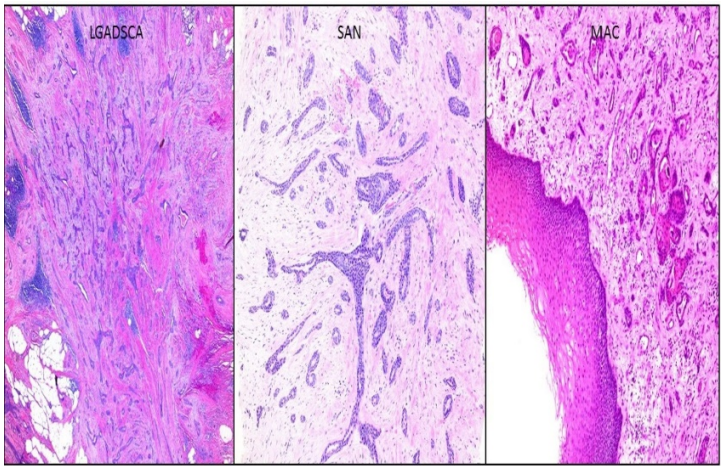
Design: A total of 16 cases, including SAN (5), LGASCA (9) & MAC (2) were identified in database (2000-2019). H&E slides and paraffin blocks of 7 cases with sufficient tumor [SAN (3), LGASCA (3) and MAC (1)] were retrieved. Diagnosis was confirmed by 2 pathologists. Tumor tissue was macrodissected, sequenced & analyzed using AmpliSeq Cancer Hotspot Panel v2 covering ~2800 COSMIC mutations across 50 cancer-related genes.

Results: Molecular findings are summarized in table 1. Variant (VAR) calls were annotated with Annovar and filtered with a custom script on several VAR metrics and annotation results. Minimum (MIN) depth of coverage for a VAR was set at 100x, MIN allele frequency at 5%, & MIN VAR quality at 20 for SNPs and 30 for indels. VAR occurring in introns/splice sites, synonymous VAR & VAR with a population frequency >1% were removed. 1/3 LGASCA samples failed extraction. Of the remaining 2 cases, 1 case showed nonsynonymous SNV in *PIK3CA* (exon21:c.A3140G:p.H1047R). None of 3 SAN cases exhibited genetic alterations. Interestingly, MAC case harbored multiple pathogenic genetic aberrations, in *KDR*, *FBXW7, JAK2*, *CDKN2A*, *TP53*. Of note, this patient died due to multiple other comorbidities. The remaining 6 patients of LGASCA and SAN are alive.

Table: Molecular findings of LGASCA, SAN and MAC

Case No	Altered Gene	Type of mutation	Variant Frequency
1. LGASC	None	N/A	N/A
2. LGASC	**PIK3CA (exon21:c.A3140G:p.H1047R)**	nonsynonymous_SNV	13%
3. LGASC	None	N/A	N/A
4. SAN	None	N/A	N/A
5. SAN	None	N/A	N/A
6. SAN	None	N/A	N/A
7. MAC	KDR	nonsynonymous_SNV	13%
	FBXW7	nonframeshift_deletion	5%
	JAK2	nonsynonymous_SNV	31%
	CDKN2A	frameshift_deletion	11%
	TP53	stopgain	12%
	TP53	nonsynonymous_SNV	10%

Figure 1 - 219



Conclusions: One case of LGASCA showed *PIK3CA* gene mutation, while none of the SAN cases showed any genetic alterations. This subtle finding could be very helpful & in turn can help delineating LGASCA from SAN, rather diagnosing them based on their location. Furthermore, MAC harbored multiple genetic aberrations (*KDR, FBXW7, JAK2, CDKN2A, TP53*) conforming with its aggressive behavior. Molecular analysis on these rare tumors with identical histomorphology can help predicting biological behavior and can potentially assist in defining these lesions.

220 L1CAM Expression in Recurrent Estrogen Positive/HER2 Negative Breast Cancer

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Disclosures: Ioana Moisini: None; Huina Zhang: None; David Hicks: None; Bradley Turner: None

Background: L1 cell adhesion molecule (L1CAM) is a 200-220 kDa transmembrane glycoprotein of the immunoglobulin superfamily, involved in neurogenesis, cell-cell interaction, synaptogenesis, myelination, and neuron survival. L1CAM is associated with poor prognosis in many carcinomas and has been proposed to be a strong prognostic factor in endometrial carcinoma; however, little is known about its expression and prognostic value in breast cancer. Triple-negative breast carcinomas have been suggested to overexpress L1CAM, associated with shorter disease-free and overall survival. Our goal is to evaluate the role of L1CAM in recurrence and metastasis of ER positive/Her-2 negative breast carcinomas.

Design: A retrospective search of the pathology database at the University of Rochester Medical Center between January 2008-December 2015 was performed to identify patients with recurrent ER positive/Her-2 negative breast carcinomas. 152 cases fulfilling these criteria were eligible. The control group included 152 cases of ER positive/Her-2 negative breast carcinomas that did not recur. Ki-67 was available in 115 cases. 36 cases (31.5%) were luminal A subtype (Ki-67 <14%), and 79 cases (68.5%) were luminal B subtype (Ki-67 ≥ 14%). Time of recurrence ranged from 1-14 years, with the predominant sites including ipsilateral or contralateral breast, chest wall, liver, lung, and bone. Immunohistochemical studies were performed on formalin-fixed paraffin-embedded tissues using the L1 mAb clone. Based on previous literature, L1CAM expression of ≥10% is considered positive. All L1CAM positive cases received adjuvant chemotherapy and hormonal therapy, with negative surgical margins.

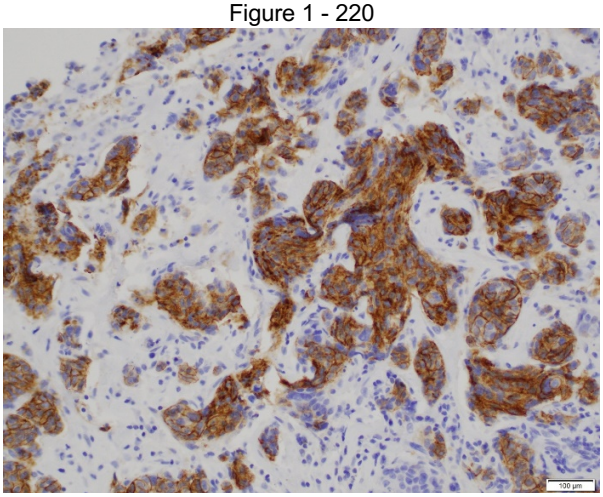
Results: L1CAM expression was found to be highly specific for recurrence (Table 1), with significant expression of L1CAM in patients that recurred compared to the control group (RR 2.048, $p < 0.05$). None of the 152 cases in the control group had positive L1CAM expression. Seven of the 152 cases that recurred had positive L1CAM expression; all of them were luminal B subtypes, with a Ki-67 proliferation index between 30% and 80%. Luminal B subtypes with positive L1CAM expression were more likely to have a higher Ki-67 expression than luminal B subtypes negative for L1CAM.

Table 1: Overall L1CAM expression, and Ki-67 expression in recurrent luminal A and luminal B subtypes

	Recurrence	No recurrence
L1CAM positive	n = 7	n = 0
Average Ki-67 expression in recurrent cases		
Luminal A (n = 0)	NA	
Luminal B (n =7)	41%	
L1CAM negative (n =297)	n = 145	n = 152
Average Ki-67 expression in recurrent cases*		
Luminal A (n =36)	6%	
Luminal B (n =72)	38%	

* Ki-67 was available in 75% (108/145) of L1CAM negative recurrent cases

Figure 1 - 220



Conclusions: Our findings suggest that L1CAM expression is specific for recurrence in luminal B subtype breast carcinomas, particularly those with higher Ki-67 expression. Additional studies with larger populations are needed to further validate these results.

221 Association between mRNA Expression of B7 Immune Checkpoints and Clinicopathological Factors in Breast Carcinoma

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Disclosures: Maria Niveiro: None; Elena Castellon-Molla: None; Gloria Peiro: None

Background: B7 immune checkpoints are transmembrane proteins that regulate T cells via co-stimulatory or co-inhibitory signals. The best-known co-inhibitory molecules are PD1, PDL1, PDL2, CD276 and CTLA4, whose deregulation in neoplastic cells can lead to the evasion of the immune response. In fact, recent studies have related these molecules to tumor progression, invasion, and metastasis in different neoplasias. In breast carcinoma (BC) the correlation of these genes expression levels with clinicopathological features has not been studied extensively.

Design: We analyzed the mRNA expression of *PD1, PDL1, PDL2, CD276 and CTLA-4* genes in a series of non-consecutive BC (n=150) including: Luminal A (25%), Luminal B/HER2- (23%), Luminal B/HER2+ (25%), HER2-enriched (8%) and Triple-Negative/Buasal-like (TN/BL) (19%). Analysis was performed by qRT-PCR using TaqMan® probes, using *PUM1* and *β-actin* as reference genes, and a pool of mRNA from healthy breast tissue as reference calibrator. Relative changes in gene expression were calculated as the fold-change by the

$2^{-\Delta\Delta Ct}$ method. Results were correlated with clinicopathological factors (age, tumor size, histological grade, lymph-vascular invasion, necrosis, immunophenotype, lymph-node and estrogen receptor –ER- status) using the χ^2 test.

Results: Patients' age average was 57 years old (range 32-94 years). Tumors showed predominantly histological grade 3 (51.3%), no necrosis (80%) or vascular invasion (64%), and lymph-node positive status (60%). Satisfactory expression in all genes was observed in 142 (94.7%) tumors. A positive correlation was found between the gene expression ($p \leq 0.02$), except for *CD276-PD1* and *CD276-CTLA4* (p=0.47 and p=0.62, respectively). High *PD1* mRNA levels correlated with lymph-node positive status (p=0.017), whereas *PDL2* and *CD276* with no vascular invasion (both $p \leq 0.018$), and *CTLA4* expression r in ER-negative tumors (p=0.001). Grade 3 tumors showed increased levels of *PDL2* and *CTLA4* (p=0.05 and p=0.001, respectively). However, there was no association between *PDL1* expression and clinicopathological factors (all p>0.05).

Conclusions: Our results in a clinical series of patients with BC show that *PD1* and *CTLA4* are associated with poor prognostic parameters, consistent with their co-inhibitory function of the immune response. Conversely, high *CD276* and *PDL2* mRNA expression may act as good prognostic biomarkers, specifically *PDL2* in a subset of high grade tumors.

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222 Genomic Profiling of Cystic Hypersecretory Carcinoma In Situ

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Disclosures: Kelly Mooney: None; Kimberly Allison: None; Robert West: None; Gregor Krings: None; Yunn-Yi Chen: None; Megan Troxell: None; Chieh-Yu Lin: None; Gregory Bean: None

Background: Cystic hypersecretory carcinoma in situ (CHCis) is a rare form of ductal carcinoma in situ (DCIS) involving cystically dilated ducts with luminal eosinophilic colloid-like secretions. Molecular characterization of CHCis has not been reported and it is not clear if this distinct histology has characteristic genetic drivers. We profiled 8 CHCis cases, including 1 which recurred 2 years later with morphologically similar invasive carcinoma, by capture-based next generation sequencing (NGS) to determine whether these tumors have shared genomic alterations.

Design: Clinicopathologic data was collected for 8 patients. DNA was separately extracted for sequencing from 8 CHCis, matched normal tissue for 7 cases, and 1 recurrent invasive carcinoma. NGS targeted exons of 479 cancer genes, 40 introns and the *TERT* promoter. Single nucleotide variants, insertions/deletions and copy number alterations (CNA) were evaluated.

Results: Seven patients were female and one was male; the average age was 56 years old (range 37-69). One-half presented with an abnormality on screening mammogram (4/8 including 2 for calcifications and 2 for masses), followed by palpable mass (3/8), and bloody nipple discharge with palpable mass (1/8). CHCis size ranged from 1.9 to 12 cm (mean 4.6). Nuclear grade was intermediate (6/8) or high (2/8) with comedonecrosis (4/8) or single cell necrosis (1/8). Six cases were ER positive. Pathogenic genomic alterations were detected in all cases. Alterations were identified in the *ERBB2/ERBB3/PIK3CA/AKT1* pathway (5/8), chromatin modifiers (4/8), *GATA3* (2/8) and *TP53* (2/8). *TP53* mutations were exclusive to cases with high nuclear grade. ER-negative CHCis had either *ERBB2* or *ERBB3* mutation. Recurrent CNA included gains of chromosome 1q (5/8) and distal 17q (4/8), as reported in conventional DCIS. The in situ and recurrent invasive carcinoma harbored shared aberrations.

Case #	Age	Sex	Nuclear Grade	ER	PR	HER2	Pathogenic Alterations	Follow-Up (months)
1	49	F	Int.	+++	+++	-	PIK3CA p.E545K, GATA3 p.S411fs, ARID1A p.R2232fs, PPM1D amplification	NED (6)
2	53	M	Int.	+++	++	nd	PIK3CA p.E545K, GATA3 p.N334fs, APC p.S559fs	NED (11)
3	37	F	High	++	++	nd	TP53 p.Y205C, p.S214W, NOTCH2 amplification	AWD (44)
4	57	F	High	+++	+	nd	TP53 p.R342*, ZNF703/GPR124/FGFR1 amplification, EMSY/PAK1/GAB2 amplification, KDM5A amplification, PPM1D amplification	NED (54)
5	58	F	Int.	+++	++	nd	AKT1 p.E17K	NED (10)
6	53	F	Int.	+	-	nd	ARID5B p.K578*, KDM6A c.619+1->T	NED (10)
7	69	F	Int.	-	-	2+ and low-amplified by FISH	ERBB2 p.S310Y, PIK3CA p.H1047R, GNAS p.R201C, PTPN11 p.S502L, PTPRK p.Q484*, FOXA1 p.P324_A325delinsAS*	NED (4)
8 (CHCis)	69	F	Int.	-	-	nd	ERBB3 p.E928G, p.V104L, SMARCB1 p.R374Q	LFU
8 (invasive CHC)	71	F	Int.	-	-	-	ERBB3 p.V104L, SMARCB1 p.R374Q	LFU

+++ >95%, ++ 10-95%, + 1-10%, - negative (<1%), nd not done, Int. intermediate, NED no evidence of disease, AWD alive with disease (metastasis of contralateral breast cancer), LFU lost to follow-up

Conclusions: CHCis shows overlapping molecular alterations with conventional DCIS. Genomic evidence supports evolution of invasion from CHCis.

223 Inter-Observer Variability in the Reporting of Extranodal Extension in Metastatic Breast Carcinoma: Results of an International Survey of Breast Pathologists

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Disclosures: Michael Moravek: None; Gabriela Oprea-Ilies: None; Stefan Pambuccian: None

Background: Lymph node metastasis is the single most important prognostic factor for breast cancer patients. In addition to the number of positive nodes and the size of the largest metastatic focus, the presence of extranodal extension has been added to the pathology report template in many institutions. Extranodal extension (ENE) is defined as tumor cells penetrating through the lymph node capsule into the peri-nodal tissue.

In the 2016 AJCC staging manual and the August 2019 version of the CAP staging synoptic reporting template, the reporting of the presence but not of the extent of extranodal extension is a required component. Recently, clinicians in an increasing number of institutions require pathologists report the extent of ENE to guide their management decision. The CAP protocol lists the extent of ENE as an optional data element; however, there is currently no consensus guideline specifying how to measure ENE for axillary nodes involved by breast cancer, and studies reported in the literature have used various ways of measuring ENE.

Design: A web-based survey containing 21 questions, 8 of which regarded microphotographs depicting lymph node sections, was distributed to an international group of breast pathologists that had published breast cancer associated research during last 10 years. Questions regarding the presence/absence of ENE, the reporting of micrometastasis versus macrometastasis in the presence of ENE, the method of measuring ENE, and demographical questions were included.

Results: A total of 65 responses were received. Responses showed that 71% of respondents had been practicing breast pathology for at least 10 years. The majority of respondents (62%) reported signing out breast pathology exclusively or almost exclusively. Overall, the results of the survey demonstrated marked interobserver variability regarding the presence of ENE and the method of reporting the extent of ENE. The questions that had most disagreement were related to the distinction between afferent lymphatic invasion and ENE, and the method of measuring ENE extent and the diagnosis of micrometastasis with ENE. Responses to selected questions are shown in figures 1 and 2.

Figure 1 - 223

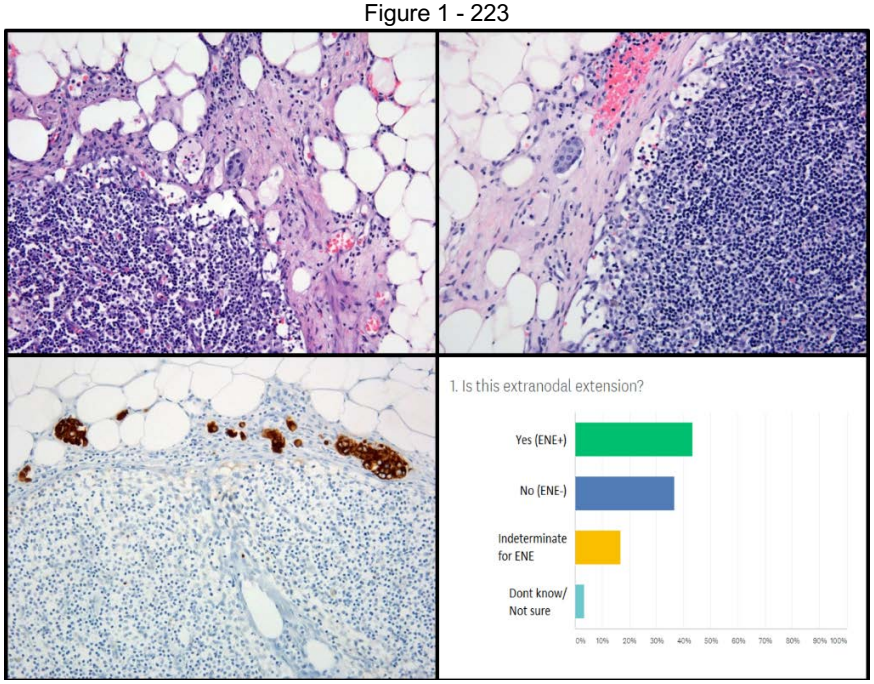
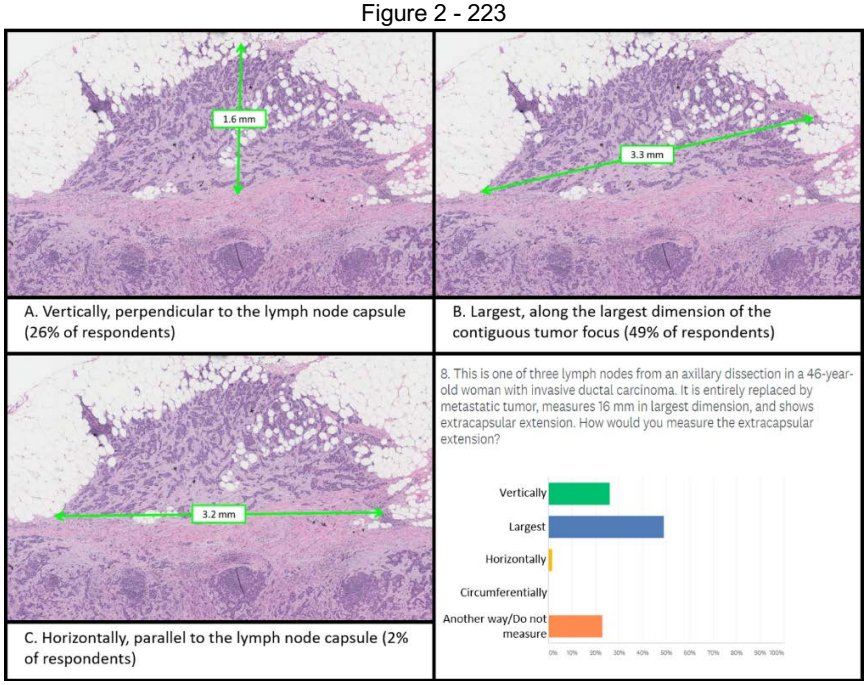


Figure 2 - 223



Conclusions: The results of this survey uncovered marked variability in the diagnosis of ENE and the reporting of its extent. Since the different methods of measuring ENE may result in differences in extent considered clinically significant (>2mm or <2mm), our findings demonstrate the urgent need for standardization of ENE reporting.

224 Unveiling the Histopathologic Spectrum of MRI-Guided Breast Biopsies: An Institutional Pathological-Radiological Correlation

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Disclosures: Gustavo Moreno: None; Julie Jorns: None

Background: MRI-guided breast biopsy has become a common adjunct in pre-surgical breast cancer work-up, is a preferred radiological technique for evaluation of indeterminate/suspicious breast abnormalities not readily visualized by conventional imaging and may be used in screening of high-risk women. No specific MRI radiologic finding definitively separates benign and malignant breast lesions; however, regional non-mass enhancement (NME) is suggestive of benign (NPV, 92%) and regional mass enhancement (ME) of malignant (PPV, 81%) etiology. Pathological-radiological MRI concordance literature is scant and inconsistent. Our goal was to evaluate the correlation between pathology proven benign and malignant lesions and MRI NME/ME lesions.

Design: Retrospective database search (1/18-12/18) was used to identify breast core biopsies from 3 hospitals that comprise our institution. Clinicopathologic features were assessed by chart and slide review. Biopsies were categorized by radiological finding (NME or ME) and into 9 benign, 1 atypical and 2 malignant pathologic groups.

Results: 2909 breast biopsies were reviewed, 232 (8%) of which were MRI-guided (figure 1). The majority of MRI biopsies were done for concurrent cancer or in evaluation of a suspicious lesion (184, 79.3%) and 48 (20.7%) for screening. Mean age was younger for MRI (52 yrs vs overall mean age of 56 yrs) and youngest for the subgroup undergoing MRI for screening (mean age 45 yrs). MRI finding was: 121 (52.2%) NME and 111 (47.8%) ME. Pathology was: 171 (73.7%) benign, 34 (14.6%) malignant and 27 (11.6%) atypia. The most common lesion seen as NME was clustered cysts with papillary apocrine metaplasia (23, 9.9%) and the most common ME lesion was fibrocystic changes NOS (29, 12.5%); however, a spectrum of pathology was seen for both NME and ME (table 1, figure 1). NME correlated with benign pathology, with a negative predictive value of 82.57% (similar to available literature). However, ME correlated poorly with malignant pathology, with a positive predictive value of 15.62%.

BENIGN		Number	total%	NME	total%	row%	EM	total%	row%
	FA	28	12.10%	11	4.70%	39.30%	17	7.30%	60.70%
	IDP	11	4.70%	7	3.00%	63.60%	4	1.70%	36.40%
	FCCNOS	50	21.60%	21	9.10%	42.00%	29	12.50%	58.00%
	CCPAM	39	16.80%	23	9.90%	59.00%	16	6.90%	41.00%
	RSL	2	0.90%	0	0.00%	0.00%	2	0.90%	100.00%
	Inflam/ID	9	3.90%	7	3.00%	77.80%	2	0.90%	22.20%
	BBTNOS	30	12.90%	21	9.10%	70.00%	9	3.90%	30.00%
	B9LN	1	0.40%	0	0.00%	0.00%	1	0.40%	100.00%
	Other B9	1	0.40%	0	0.00%	0.00%	1	0.40%	100.00%
ATYPICAL	Atypia	27	11.60%	12	5.20%	44.40%	15	6.50%	55.60%
MALIGNANT	In situ	14	6.00%	11	4.70%	78.60%	3	1.30%	21.40%
	Invasive	20	8.60%	8	3.40%	40.00%	12	5.20%	60.00%
	Total	232		121			111		
	Abbreviations: NME, non-mass enhancement; EM, enhancing mass; FA, fibroadenoma; IDP, intraductal papilloma; FCCNOS, fibrocystic changes NOS; CCPAM, cluster cysts with papillary apocrine metaplasia; RSL, radial sclerosing lesions; inflame/ID, inflammatory/infectious lesions; BBTNOS, benign breast tissue NOS; B9LN, benign lymph node; Other B9, other benign.								

Figure 1 - 224

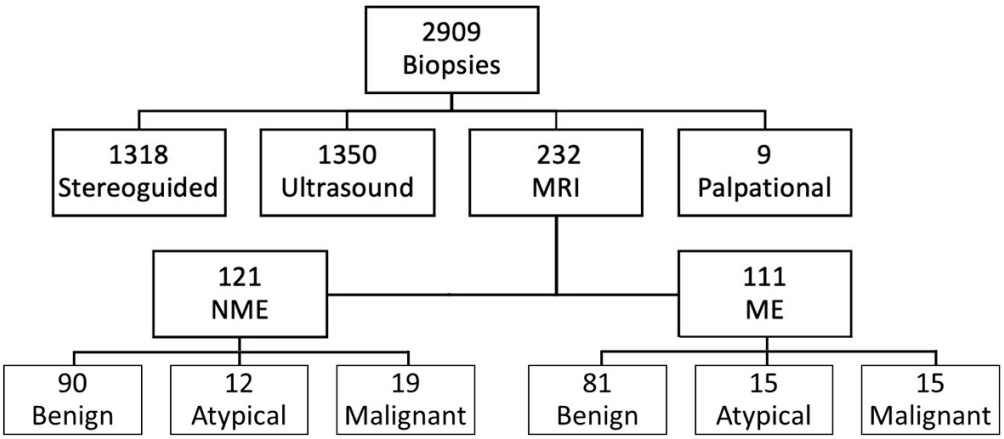
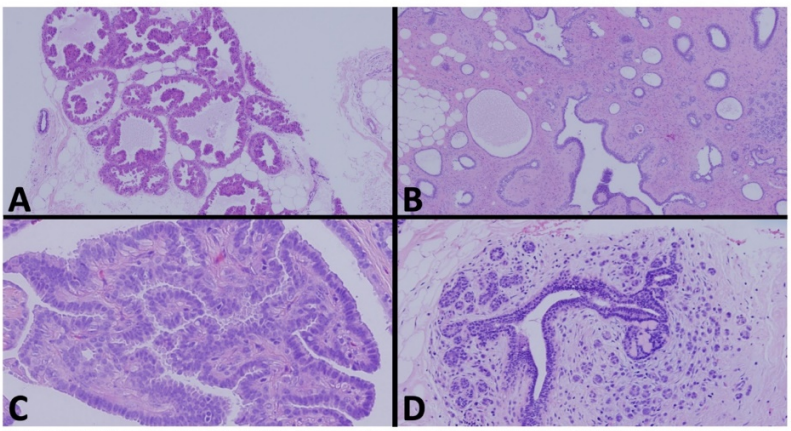


Figure 2 - 224



A: Cluster cysts with papillary apocrine metaplasia, 4x. B: Fibroadenoma, 4x. C: Intraductal papilloma, 20x. D: Invasive lobular carcinoma, 10x.

Conclusions: MRI-guided breast biopsy is a relatively new alternative to localize and sample suspicious lesions not easily seen by other imaging techniques. NME is frequently associated with benign lesions. However, EM does not appear to be discriminatory of malignant lesions as previously suggested.

225 Solid Papillary Carcinoma and Encapsulated Papillary Carcinoma of the Breast: Clinical-Pathologic Features and Basement Membrane Studies of 50 Cases

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Disclosures: Sarah Morgan: None; Jessie Wu: None; James Cotton: None; Gulisa Turashvili: None

Background: Solid papillary carcinoma (SPC) and encapsulated papillary carcinoma (EPC) are rare and poorly understood subtypes of papillary carcinoma with distinct features and diagnostic challenges. Despite the lack of myoepithelial cells similar to invasive, EPC and SPC are considered in situ lesions based on favorable clinical outcomes. We aimed to describe clinical-pathological features including basement membrane (BM) studies in these tumors.

Design: We retrospectively identified specimens with diagnosis of SPC or EPC in 2000-2019. Histology slides were reviewed and clinical-pathologic variables were recorded. Immunohistochemical stains for BM (collagen IV) and myoepithelial (p63, SMM) markers were performed.

Results: The cohort consists of 23 SPCs and 27 EPCs. Surgical procedures included 5 total mastectomies (TM) and 18 breast-conserving surgeries (BCS) for SPC, and 4 TM and 23 BCS for EPC. All patients were female except 1 male patient with SPC. The median age was 71 years (49-93). Clinical presentations included mass in 19 cases and nipple retraction/discharge in 5 cases. Of 23 SPCs, 2 (21.7%) were pure SPC, 4(17.4%) associated with ductal carcinoma in situ (DCIS), 6 (26.1%) with invasive carcinoma (5 invasive ductal carcinoma no special type (IDC NST), 1 mucinous carcinoma), while 8 (34.8%) were considered invasive due to infiltrative growth. Of 27 EPC patients, 9 (33.3%) were pure EPC, 12 (44.4%) associated with DCIS, and 6 (22.3%) with IDC. The median tumor size was 1.4 cm (0.1-16) and 2.2 cm (0.7-5.9) for invasive and in situ lesions, respectively. All tumors were positive for hormone receptors and negative for HER2. Myoepithelial cells were absent in 20 cases and focally present in 30 cases. Collagen IV was absent in all invasive lesions but present in all EPCs and 20 SPCs, including invasive SPC. Of 26 patients who underwent sentinel lymph node biopsy, 1 patient with EPC+IDC had a positive node. Of 31 patients with available follow-up (median 35 months, 1-85), 1 SPC (4.3%) and 1 EPC (3.7%), both associated with IDC, developed local recurrence. No distant recurrence or deaths were observed.

Conclusions: Our study confirms that SPC and EPC are special types of breast cancer with low (4%) recurrence rates. SPC is more commonly associated with invasive carcinoma or considered invasive compared to EPC (61% vs 22%). The presence of BM material in most cases indicates that these tumors may represent in situ lesions evolving into low-grade invasive malignancy.

226 Correlation of High Resolution Tomographic Images and Histopathology in Breast Lumpectomy Surgical Margin Assessment

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Disclosures: Jeffrey Mueller: None; Xiao Han: *Stock Ownership*, Clarix Imaging Corporation; Alexis Snyder: None

Background: Positive surgical resection margins has been a long-standing issue in breast-conserving surgery and is the leading cause for re-excision procedures. Currently, faxitron imaging is the most commonly employed method of intra-operative surgical margin assessment. However, this method of assessment is subject to limitations such as workflow interruption or suboptimal sensitivity for positive margin detection. 3D imaging experiments with lumpectomy specimens show that invasive carcinoma as well is microcalcifications can be clearly visualized. In this study we use volumetric tomographic images to assess tumor dimensions and surgical margin status and correlate the findings with the final histopathology.

Design: We performed high resolution tomographic images of 17 lumpectomy specimens before the specimens were grossed or triaged. A breast radiologist reviewed the images without knowledge of the pathologic findings. The distance of the tumor or microcalcifications was assessed and recorded. A surgical margin was considered positive if microcalcifications or tumor were present within 1.0 mm from any surgical margin. Each of the six surgical margins was assessed separately. The results were than correlated with the histopathologic findings.

Results: Results show that with the volumetric images, the radiologist was able to achieve 100% sensitivity and 92% specificity for identifying positive margins. In conclusion, we demonstrated that the volumetric imaging device can yield high-resolution 3D images of lumpectomy specimens within a clinically acceptable timeframe, thus can potentially be used for intra-operative margin assessment.

There were a total of 102 surgical margins assessed (6x17).

3D imaging identified 13/13 positive surgical margins.

3D imaging identified 86/95 negative surgical margins.

Conclusions: We demonstrated that the volumetric imaging shows high correlation with histopathology in terms of surgical margin assessment and may potentially be useful in the gross room or surgical site for intra-operative assessment of surgical margins.

227 Comparing Oncotype DX with Magee Equation 2: Analysis from an Urban Public Hospital

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Disclosures: Patrick Mullane: None; Marina Mosunjac: None; George Birdsong: None; Uma Krishnamurti: None

Background: Oncotype DX (ODX) is a commercial genetic assay used to predict the risk of breast cancer (BC) recurrence. However, its cost is a financial burden for patients and hospitals with limited financial resources. “Magee equation 2” (ME2) is a linear regression analysis designed as a free alternative tool to ODX using only standard histopathologic factors and predictive markers routinely reported in

BC pathology reports (tumor size, Nottingham score, ER, PR, and HER2 status). Here, we compare ODX with ME2 recurrence scores in patients at an urban public hospital.

Design: 106 patients with ER positive BC with available ODX reports and prerequisite histopathologic data were reviewed. ME2 results were calculated using the UPMC calculation https://path.upmc.edu/onlineTools/mageeequations.html. Scores were grouped into three risk categories: 0<18 (low), 18 to < 31 (intermediate), and > 31 (high). Concordance and Pearson correlation calculations were performed for comparisons.

Results: Overall concordance between ODX and ME2 was 51.5% with a correlation of 0.58. The concordance for low, intermediate, and high risk groups was 72%, 37%, and 22%, respectively. There was no two-step discordance between ME2 and ODX. All ME2 high risk scores were also ODX high risk. ME2 had a higher proportion of intermediate score cases (51 vs. 33). ME2 scores were lower than ODX in cases of ER/PR discordance and grade 1 tumors, while they were higher in larger T2/T3 tumors and in smaller grade 3 tumors. Concordance of ODX and IHC/FISH analysis for ER, PR, and HER2 status was 96%, 86%, and 93%, respectively. 4 ER discrepant cases were only IHC+; of 15 PR discrepant cases, 12 were only IHC+ and 3 were only ODX+. Of 7 HER2 discrepant cases, 4 were IHC+ and 3 were IHC and FISH equivocal.

Conclusions: Our cohort demonstrated good correlation between ODX and ME2 scores. Concordance was highest in low risk groups, and all ME2 high risk cases were ODX concordant. Although infrequent, IHC was more sensitive for ER/PR status and disagreement on this was associated with discordant risk group classification. These findings demonstrate ME2 intermediate risk score cases can be selectively sent for additional ODX testing. Such a practice would be cost saving without compromising clinical care.

228 Utility of HER2 Immunohistochemistry in Clarifying HER2 Status in Breast Cancer with HER2:CEP17 Ratio <2 and Average >/= 4.0 and <6.0 HER2 Signals per Tumor Cell by Fluorescence in-situ Hybridization (Focused Update Group 4)

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Disclosures: Kristen Muller: None; Jonathan Marotti: None; Laura Tafe: None

Background: In May 2018, the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) published a clinical practice guideline focused update on Human Epidermal Growth Factor Receptor 2 (HER2) testing in breast cancer (PMID: 29846104). Prior to this update, breast cancers with an average of ≥4.0 and <6.0 *HER2* signals per tumor cell and a *HER2*/CEP17 ratio <2.0 were classified as ISH "Equivocal" for HER2. Per the 2018 guideline update, additional workup with HER2 immunohistochemistry (IHC) is recommended to arrive at a definitive HER2 status for tumors falling within this category ("group 4").

Design: The aim of our study was to investigate the utility of HER2 IHC in the evaluation of breast tumors with group 4 *HER2* ISH results: tumors with an average of ≥4.0 and <6.0 *HER2* signals per tumor cell and a *HER2*/CEP17 ratio <2.0. Our lab uses dual-probe FISH as the primary method for determining HER2 status on all newly diagnosed invasive breast cancers. All invasive breast cancers that underwent HER2 FISH testing from 5/2018 - 9/2019 were included. HER2 FISH and IHC was interpreted according to the ASCO/CAP 2018 guidelines.

Results: Of 613 breast tumors tested with HER2 FISH, 26 (4.2%) were categorized as group 4 (mean *HER2* ratio = 1.5 ± 0.2 with 4.6 ± 0.5 average *HER2* signals per cell). The majority of cancers were intermediate and high grade (96%), ER-positive (88%) invasive ductal carcinomas (88%). The subsequent HER2 IHC result were as follows: equivocal (IHC 2+) in the majority (n=24) and negative (IHC 1+) in one single case. The remaining case showed HER2 heterogeneity on the biopsy with <10% HER2-positive cells; repeat HER2 on the excision specimen confirmed positive HER2 status. Per guidelines, cases with 2+ HER2 IHC were reported as "Negative" with a comment. None of the patients received treatment with an anti-HER2 agent. Oncotype DX was performed on 12 cases, per oncologist request: HER2 was reported as "Negative" in 11 and "Equivocal" in one. HER2 FISH and IHC was repeated on nine subsequent excision specimens; however, this did not reclassify HER2 status, except in the single aforementioned case with heterogeneity.

Conclusions: Unless HER2 heterogeneity was present, the HER2 IHC was equivocal in all group 4 cases. These results suggest that adding IHC likely doesn't contribute to the HER2 classification of group 4 breast tumors, and perhaps such cases should be considered "Negative" based on the initial FISH results.

229 Fibroepithelial Lesions Classification Using Deep Neural Convolutional Networks, a Focus on Cellular Fibroadenomas

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Background: Convolutional neural networks (CNNs) are a main component of deep learning modules, and have been widely employed to analyze visual imagery. They have achieved physician-level accuracy at a broad variety of diagnostic tasks. The distinction between cellular fibroadenoma and phyllodes tumor frequently poses a diagnostic challenge to practicing surgical pathologists. In this study we aim to investigate the use of CNNs to classify fibroepithelial lesions with a focus on cellular fibroadenomas.

Design: Whole slide image scanning was done on all glass slides of 180 fibroepithelial lesion cases (46 benign phyllodes tumors, 27 borderline phyllodes tumors, 24 malignant phyllodes tumors, 31 fibroadenomas with high cellularity, 43 fibroadenomas with moderate cellularity and 9 fibroadenomas with low cellularity). 20 images from each case were taken. Eighty percent of the cases from each category were used to train the model using transfer learning on Inception V3 through TensorFlow. Twenty percent of the cases from each category were separated as a test group, and not used in the training process: 50 images each from 5 cases per category (benign, borderline and malignant phyllodes tumor and fibroadenoma with high cellularity), and 50 images from 8 cases of fibroadenomas with moderate cellularity. The test-set images were subsequently classified by the trained model in a binary fashion (fibroadenoma vs. phyllodes tumor). Sensitivity and specificity were used to quantify the performance of the CNN as diagnostic test differentiating cellular fibroadenomas vs other categories.

Results: The model had a sensitivity of 98% and specificity of 62% in recognizing malignant phyllodes tumors vs. fibroadenomas with high cellularity. Comparing borderline phyllodes tumors vs. fibroadenomas with high cellularity, sensitivity was 100% and specificity was 62%. Lastly, benign phyllodes tumors vs. fibroadenomas with high cellularity resulted in a sensitivity of 60% and specificity of 62%.

Conclusions: The CNN was able to differentiate malignant and borderline phyllodes tumors from cellular fibroadenomas with high sensitivity. Sensitivity was; however, moderate in differentiating benign phyllodes tumors from cellular fibroadenomas. Additionally, the specificity achieved in differentiating cellular fibroadenomas from all phyllodes tumor categories was moderate.

230 Overexpression of α-Methylacyl-CoA Racemase (AMACR) in Apocrine Carcinomas of Breast

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Disclosures: Harumi Nakamura: None

Background: α-methylacyl-CoA racemase (AMACR), also known as P504S, plays an important role in mitochondorial and peroxisomal beta-oxidation of branched-chain fatty acid. Overexpression of AMACR is highly sensitive and specific marker for prostatic carcinoma and the antibody of AMACR is widely used. In apocrine carcinoma of the breast, increase in mitochondria and overexpression of the genes related to lipid metabolism are known. We investigated the utility of AMACR antibody for differential diagnosis of apocrine carcinoma and the condition of mRNA level of AMACR in apocrine carcinoma for therapeutic application.

Design: In this study, we investigated expression of AMACR protein in 67 breast carcinomas (23 apocrine carcinomas, 10 apocrine ductal carcinomas in situ (DCIS), and 34 non-apocrine carcinomas) and 5 apocrine metaplasia of normal breast tissue by immunohistochemical analysis. Conveniently, the scoring used Allred score: Total score (TS) = Proportion score (PS) + Intensity score (IS). mRNA levels of AMACR in 5 apocrine carcinomas showing high expression of AMACR protein (TS8) and 5 non-apocrine carcinomas showing no expression of AMACAR protein (TS0) were assessed by microdissection and real time PCR (RT-PCR).

Results: Immunohistochemical analysis using a monoclonal antibody for AMACR (P504S) demonstrated that high TS (7 or 8) of AMACR was present in 12 of 23 (52.2%) of apocrine carcinomas and in 7 of 10 (70.0%) of apocrine DCISs, and there was no AMACR negative case. In addition, benign apocrine metaplasia was also positive for AMACR. No expressing AMACR was in normal ducts and lobules. Twenty seven of 34 (79.4%) of non-apocrine carcinomas were negative for AMACR and none of them showed TS8. We are analyzing the mRNA levels of AMACR at present.

PS+IS=TS	0+0 =0	1+1= 2	2+1= 3	2+2= 4	2+3= 5	3+1= 4	3+2= 5	3+3= 6	4+1= 5	4+2= 6	4+3= 7	5+1= 6	5+2= 7	5+3= 8	Total
Apocrine ca	0	0	0	2	1	2	2	1	1	2	2	0	0	10	23
Apocrine DCIS	0	0	0	2	0	0	0	1	0	0	1	0	2	4	10
NOS	27	0	1	0	1	0	2	0	0	1	0	0	2	0	34
Apocrine metaplasia	0	0	0	0	0	0	1	1	0	1	1	0	0	1	5
	27	0	1	4	2	2	5	3	1	4	4	0	4	15	72

Conclusions: Our findings suggest that AMACR antibody is a new useful marker to distinguish apocrine carcinoma from non-apocrine carcinomas, especially triple negative breast carcinomas.

231 "Growing Fibroadenoma": A Retrospective Review of 21 Cases at a Tertiary Healthcare Institution

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Background: Fibroadenoma (FA) is common benign breast tumor which is often conservatively followed with imaging modalities. In cases of growth of lesions, core needle biopsies (CNB) are often performed to rule out the possibilities of phyllodes tumor (PT) or mimics such as tumors with "circumscribed growth". The study is to identify features of those "growing FAs" that are re-classified as PT (false negative core biopsy).

Design: Twenty-one tumors in twenty patients with radiologic documentation of "growing FA" and pathology diagnosis of "FA" or "favoring FA" between 2012 and 2019 were identified. H&E slides for CNB and follow up re-biopsy or excision were reviewed. Various histopathologic, radiologic and clinical parameters were recorded.

Results: All patients were female with mean age of 33.3 (range: 17-52) and average BMI of 28.6kg/m^2 (range: 20.7-45.8). 15/21 cases had pathologic diagnosis of FA on CNB. In 5/21 cases CNB were called fibroepithelial lesions (FEL) favoring FA. Most cases had follow up excision (16/21). There were 7/16 excisions upgraded to benign PT and 8/16 remained FA. One FEL was juvenile papillomatosis on excision. No borderline or malignant PT was found on excision. The average size of the mass was 3.9cm (range: 1-10). There was no difference in the size between FA versus PT on excision (t test p=0.42). The average size increase of the mass was 0.9mm/month and there was no difference between FA versus PT (p=0.92). However, among 11 patients with age <=30, only 1 upgraded to PT; in contrast, 6 of 10 patients aged >30 were upgraded to PT. The average mitotic rate for PT including false negative cases was higher (2.7/HPF) than that of FA (0.8/HPF), p=0.028. All lesions including PT had circumscribed borders. Four PTs were initially called FA on CNB (false negative cases). Comparison of the biopsy and excision found all cases were due to the variable cellularity of the tumor, which is a feature of PT but can be a sampling issue on CNB. There were no statistical differences in the mass size and size increase rate between the false negative cases and the rest (p>0.05).

Conclusions: The false negative rate (pertaining to FA on CNB and PT on excisions) is 4/16 (25%) on clinico-radiologic impression of a "growing FA". Older age is the most significant factor for "growing FA" to be upgraded. Mitotic rate appears to a superior indicator of PT compared to circumscription, stromal cellularity, radiology mass size and size increase rate in our study.

232 Extramammary Metastases to the Breast: A Series of 17 Cases with Clinicopathologic Characterization

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Disclosures: Gahie Nam: None; Evgeny Yakirevich: None; Yihong Wang: None

Background: Metastasis of extramammary malignancy to the breast is rare (0.3-2.7%). The major problem is to differentiate primary from metastatic extramammary neoplasms. Misdiagnosis as a primary breast cancer even in cases with a medical history of another primary cancer is common. The purpose of the study was to review the clinicopathologic and radiologic characteristics of extramammary metastases to the breast.

Design: Pathology databases were searched for breast biopsies or excisions with extramammary metastasis to the breast from 2001 to 2019 excluding primary breast skin lesions and lymphomas. Seventeen patients with metastatic breast lesions were retrieved. Clinical, radiologic and follow-up data were recorded.

Results: Sixteen patients were females and 1 male with mean age of 57 (range: 25-83). The diagnosis was established on core needle biopsies in 15/17 cases, 1 excisional biopsy and 1 lumpectomy. The average size of the metastases was 1.9 cm (range: 0.2-5.7). The most common tumor type that metastasized to the breast was melanoma (24%) (Table). Median time interval from the initial diagnosis of extramammary tumor to breast metastasis was 2.5 years (range: 1-40). 16/17 patients had known history of primary extramammary cancer prior to the breast lesion. In one case, breast neuroendocrine tumor was the initial manifestation which led to clinical workup with CT finding of a terminal ileum mass with additional metastatic sites including liver, peritoneum and lymph nodes. 9 of 16 patients (56%) had evidence of metastases outside the breast which was known before the breast biopsy. In remaining 4 of 16 patients the metastatic disease was first identified by biopsy of a "breast mass" and after confirmation of the metastatic disease with breast involvement, additional metastatic sites were identified. Only one patient had an isolated metastasis from ovarian high-grade serous carcinoma. Eight patients with follow-up information showed persistent metastatic disease and one patient passed away with residual disease.

Table: Clinicopathologic and Radiologic Features of Breast Metastatic Lesions from Extramammary Neoplasms (n=17)

Pathologic Diagnosis of Primary Neoplasm Site	Clinical Characteristics	Radiologic Findings
Melanoma: 4 (24%) Lung: 3 (18%) Leiomyosarcoma: 3 (18%) Ovaries: 2 (12%) Endometrium: 1 (6%) Esophagus: 1(6%) Pleomorphic rhabdomyosarcoma: 1(6%) Prostate: 1 (6%) Neuroendocrine tumor (Gastrointestinal): 1 (6%)	Known history of primary extramammary carcinoma prior to breast lesion (n=17): • Known history: 16 (94%) • No prior history: 1 (6%)	Mean breast mass size (n=11): • 2.0 cm (range: 0.2-5.7)
		Focality of breast lesion: • Unifocal: 10 (59%) • Multifocal: ○ Bilateral: 3 (18%) ○ Unilateral: 1 (6%)
	Presenting symptom of breast lesion (n=12): • Palpable mass: 5 (42%) • Screening mammogram: 4 (33%) • Found during follow up of known extramammary cancer: 2 (16%) • Work up of other cancer: 1 (8%)	
		Presence of microcalcification: 1/12 (83%)
	Presence/ absence of other metastases (n=16): • Present other metastatic sites (known before breast lesion): 9 (56%) • Present other metastatic sites (discovered after breast lesion): 4 (25%) • Breast only: 1 (6%) • Unknown: 2 (13%)	
		Presence of enlarged axillary lymph node: 1/12 (83%)

Conclusions: Pathologic examination and clinical history are the most helpful guide to differentiate the primary breast cancer from metastatic spread. Metastasis to the breast from an extramammary neoplasm usually indicates disseminated metastatic disease and a poor prognosis. Breast metastasis can be initial presentation of extramammary primary and an accurate diagnosis of breast metastases, differentiating primary from metastatic breast carcinoma is important for proper management.

233 Sensitivity and Negative Predictive Value of Axillary Lymph Node Core Biopsy in Pre-Operative Staging of Breast Cancer

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Disclosures: Alia Nazarullah: None; Diane Trang: None; Andrea Agualimpia Garcia: None; Lubna Alattia: None; Sara Ortiz-Romero: None; Sarah Hackman: None; Daniel Mais: None

Background: Pre-operative sampling of abnormal or suspicious axillary lymph nodes provides valuable prognostic information and guides treatment planning in breast cancer. Such lymph nodes are typically sampled by core needle biopsy (CNB) performed under manual or radiologic guidance. The reported sensitivity of axillary lymph node biopsies in this context has varied widely (27-94%) because of different radiologic criteria used. Few studies have looked at the negative predictive value of such biopsies. The purpose of this study is to analyze the sensitivity and negative predictive value of biopsies in this setting in order to better inform clinical decisions made on the basis of these diagnoses.

Design: We retrospectively identified all core needle biopsies (CNB) of axillary lymph nodes performed at our institution as part of the preoperative evaluation for breast cancer from 2015 to 2019. Cases were included only if follow-up mastectomies with lymph node sampling were available. Electronic medical records were reviewed to collect epidemiologic information, clinical findings, radiologic findings, biopsy diagnosis, and resection findings. These data were statistically analyzed.

Results: A total of 105 cases were included in the study, of which 77 cases (73%) showed metastatic carcinoma and 28 cases (27%) were negative for tumor. 13 cases (12%) were false negative on CNB and showed metastatic carcinoma on excision. The sensitivity and negative predictive value of preoperative axillary CNB in our study are 86% and 54% respectively. On further analyzing the true positive (TP) and false negative (FN) CNB categories, the average size of metastatic focus on excision is 16.7 mm in TPs and 7.3 mm in FNs ($p<0.05$). The average number of lymph nodes involved by tumor on excision is 6 in TPs and 2 in FNs ($p<0.05$). The key pathologic characteristics of the TP and FN categories are listed in Table 1.

Table 1. Characteristics of True Positive and False Negative Axillary Lymph Node Core Biopsies.

	Axillary lymph node core biopsy		P value*
	True Positive (Biopsy positive/Excision positive)	False negative (Biopsy negative/ Excision positive)	
Age range of patients	26 – 78 yrs. (mean: 53 yrs.)	35 - 72 yrs. (mean: 50 yrs.)	
Size of largest metastatic focus on excision	1 – 55 mm (mean: 16.7 mm)	0.5 - 18 mm (mean: 7.3 mm)	<0.05
Number of lymph nodes involved by tumor on excision	1-29 (mean: 6)	1-4 (mean: 2)	<0.05

*Two sample t-test assuming unequal variances

Conclusions: Pre-operative axillary lymph node CNBs have good sensitivity but low negative predictive value. Although the negative predictive value is low, the false negative CNB cases show smaller and low volume axillary nodal disease compared to the biopsy positive cases. In the current era, axillary lymph node dissection may not be indicated in many of these false negative cases with low volume disease. Additional radiologic findings that may improve negative predictive value requires further study.

234 Discordant Mitotic Score Rate in Histologic Grading of Invasive Ductal Breast Cancer

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Background: Histopathological grading in invasive breast cancer provides valuable prognostic and predictive information. The Nottingham score is widely used, but the mitotic rate score is somewhat problematic, having demonstrated the lowest level of inter-observer agreement

among the three scores and the highest susceptibility to sampling error. Furthermore, various authors have noted threshold effects at lower mitotic counts and suggested re-assessment of the cut-off points. In this study we sought to evaluate the rate of discordance between mitotic score, as compared to differentiation and pleomorphism scores, particularly in cases with a histologic score of 5 (grade 1) or 7 (grade 2) whose overall grade might be affected by a change in mitotic score.

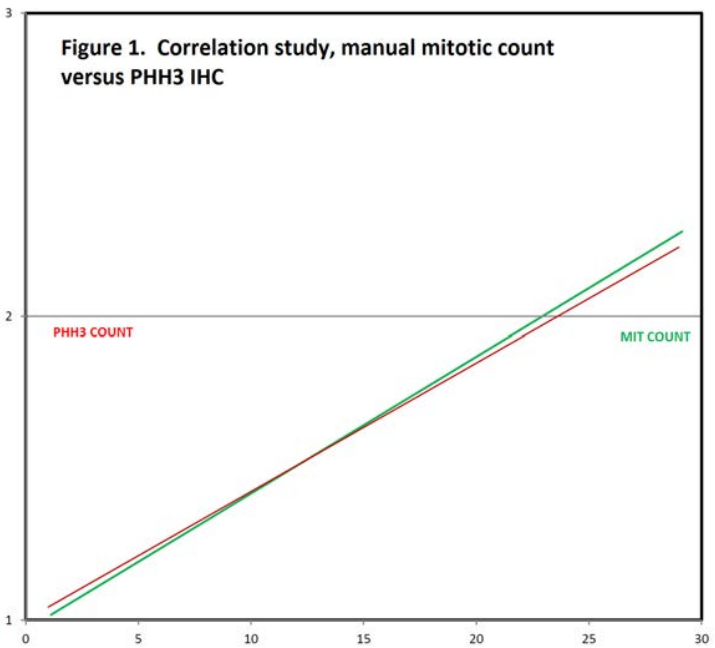
Design: We retrospectively identified all invasive breast carcinomas diagnosed over a 7-year period, 2008 to 2014, at two separate institutions. We excluded cases with the diagnosis of invasive lobular carcinoma or invasive carcinoma with lobular features, as well as grade 3 and multifocal tumors. We defined discordant cases as those in which (1) mitotic score was lower than one or both other scores and (2) if mitoses had been scored differently, a higher overall grade would be assessed (table 1). We collected follow-up clinical data, multigene predictive scores, and Ki67 indices in order to statistically analyze correlation with original and altered tumor grades. Lastly, to validate the accuracy of the original mitotic scores, a correlation study was performed with both Ki67 and PHH3 immunohistochemical stains.

Results: We identified 1230 breast core biopsies positive for carcinoma during the study period; after exclusion criteria were applied, 633 cases remained. Of these, 232 fulfilled criteria for discordance as defined above. Thus the overall discordant rate was 23.7% of all malignancies diagnosed by breast core biopsy and 47.5% of all grade 1/2 unifocal invasive ductal carcinomas. Correlation with PHH3 immunohistochemical staining was strong, with Spearman coefficient (ρ) of 0.79 (95% CI 0.56 to 0.89). Furthermore, cases given a mitosis score of 1 had mean Ki67 of 18%, and cases given mitosis score of 2 had mean 27% (p=0.0001).

Table 1. Discordant grades and scores

Overall grade	Overall score	Tubule formation	Nuclear atypia	Mitotic rate
2	7	3	3	1
2	7	3	2	2
2	7	2	3	2
1	5	2	2	1
1	5	3	1	1
1	5	1	3	1

Figure 1 - 234



Conclusions: Mitotic rate scores are discordant with scores for tubular differentiation and nuclear pleomorphism in a significant proportion of grade 1/2 invasive ductal carcinomas. The effect of this tendency on accurate prognostication, and the propriety of current thresholds, requires further study.

235 Genetic Underpinnings of Hybrid Tumors of Adenoid Cystic Carcinoma and Adenomyoepithelioma of the Breast - A Variant of Adenoid Cystic Carcinoma?

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Disclosures: Tomo Osako: None; Satoko Baba: None; Hiroko Nagano: None; Takayuki Ueno: None; Shinji Ohno: None; Futoshi Akiyama: None; Kengo Takeuchi: None

Background: Adenoid cystic carcinoma (ACC) and adenomyoepithelioma (AME) of the breast are both rare epithelial-myoepithelial tumors. ACC is genetically characterized by *MYB-NFIB* or *MYBL1-NFIB* gene fusions, while AME is characterized by *HRAS* Q61 mutations for estrogen receptor (ER)-negative cases. Although ACC and AME are histologically and genetically distinct tumors, "hybrid carcinomas" that show mixed patterns of ACC and epithelial-myoepithelial carcinoma, a counterpart of AME, have been reported in salivary gland tumors. We aimed to elucidate occurrence of hybrid tumors of ACC and AME of the breast and their genetic underpinnings.

Design: To identify the hybrid tumors of ACC and AME, we reviewed histology of 15 and 14 cases originally diagnosed as ACC and AME, respectively, resected at the Cancer Institute Hospital (Tokyo, Japan) between 1961 and 2018. Split fluorescence *in situ* hybridization (FISH) assay for *MYB*, *MYBL1*, and *NFIB* and fusion FISH assay for *MYB-NFIB* and *MYBL1-NFIB* were performed using a representative section of the tumors. Direct sequencing of *HRAS* Q61 mutations of the PCR-amplified DNA from the macrodissected tumors were also performed. ER status was determined by immunohistochemical staining.

Results: Of the 15 originally-diagnosed ACC, 3 tumors showed both ACC and AME histology and transitional and intermediate histology between ACC and AME. Of the 14 originally-diagnosed AME, all tumors had only AME histology.

Of the 12 pure ACC cases, 11 (92%) harbored *MYB/MYBL1* aberrations. Of the 14 pure AME, 2 (50%) of the 4 ER-negative AME (no PCR amplification in 1 cases), and 1 (11%) of the 9 ER-positive AME showed *HRAS* Q61 mutations, but none harbored *MYB/MYBL1* aberrations.

In 2 of the 3 hybrid tumors, *MYB-NFIB* or *MYBL1-NFIB* fusions were detected in both ACC and AME parts, while no *HRAS* Q61 mutations were detected in both parts. The remaining 1 case showed no *MYB/MYBL1* aberrations and no *HRAS* Q61 mutations in both parts.

Original diagnosis	Review diagnosis	No.	*MYB/MYBL1* aberration	*HRAS* Q61 mutation
ACC	ACC	12	11/12 (92%)	-
	Hybrid ACC and AME	3	2/3 (67%)	0/3 (0%)
AME	AME	14	0/14 (0%)	3/13 (23%)
	ER(-)	(5)	0/5 (0%)	2/4 (50%)
	ER(+)	(9)	0/9 (0%)	1/9 (11%)

Figure 1 - 235

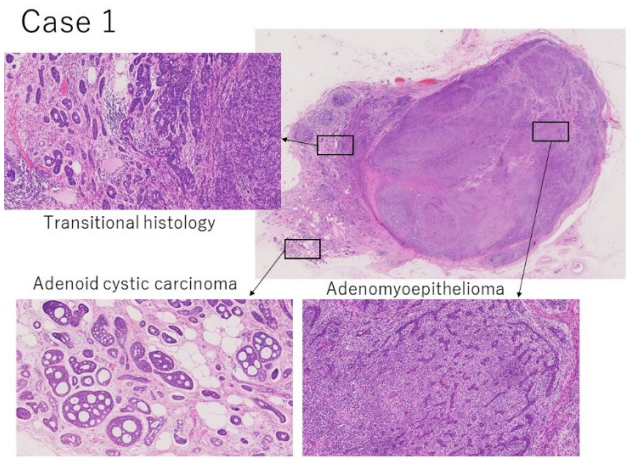
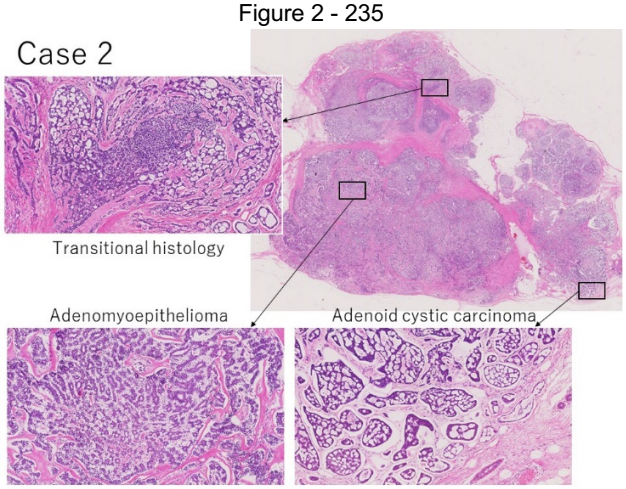


Figure 2 - 235



Conclusions: We confirmed that hybrid tumor of ACC and AME also occurs in the breast. At least, a part of the hybrid tumors of the breast should be classified as a morphological variant of ACC, because in the present study 2 of the 3 hybrid tumors were evidenced to harbor *MYB/MYBL1-NFIB* fusions in both ACC and AME parts. Meanwhile, further genetic analysis is needed to elucidate genetic underpinnings for the hybrid tumors that lack *MYB/MYBL1* aberrations and *HRAS* Q61 mutations.

236 Genomic Analysis of Multifocal Ductal Carcinoma In Situ and Synchronous Invasive Ductal Carcinoma

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Background: Ductal carcinoma *in situ* (DCIS), a non-obligate precursor of invasive breast cancer, and synchronously identified invasive ductal carcinoma of no special type (IDC-NST) are often clonally related. In this proof-of-principle study, we sought to define whether different foci of DCIS would be uniformly clonally related to the synchronously diagnosed IDC-NST.

Design: A central histopathologic review was conducted and estrogen receptor (ER)/HER2 status was determined as per the ASCO/CAP guidelines. Synchronous DCIS (n=4) and IDC-NSTs (n=3) from 2 patients with multifocal DCIS were separately microdissected and subjected to whole exome sequencing. Somatic genetic alterations, mutational signatures and clonal decomposition were conducted using validated bioinformatics methods.

Results: Multifocal DCIS 'Case 2' arose in a *BRCA1* (E23Vfs*17) germline carrier, and included 2 DCIS (2DCISA and 2DCISB) and 2 IDC-NSTs (2IDCA and 2IDCB) in the same breast quadrant, all ER-/HER2- and of grade 3. The 2 DCIS and the 2 IDC-NSTs showed loss of heterozygosity (LOH) of *BRCA1,* a dominant signature 3 (HRD) and were clonally related. The 4 lesions shared a hotspot *PIK3CA* (H1047L) and a frameshift *TP53* mutation with LOH. A minor subclone of 2DCISA harboring a *KDM5C* mutation became dominant in 2DCISB, suggesting that 2DCISB stemmed from 2DCISA. 2IDCA and 2IDCB harbored private mutations in *GNAS* and *SOX2*. Multifocal DCIS 'Case 5' included a grade 2 DCIS (5DCISA) and IDC-NST (5IDC) in the same quadrant, and a grade 3 DCIS (5DCISB) in a different quadrant, all ER+/HER2-. 5DCISB was genetically unrelated to the other lesions and had a *GATA3* P424Afs*82 frameshift mutation and a *TP53* splice site mutation with LOH. 5DCISA and 5IDC were clonally related and shared a *GATA3* P408Afs*99 frameshift and *XPO1* splice site mutations. 5IDC harbored a private *SPEN* mutation and a 15q11.1-q26.1 amplification (*NUTM1* and *IDH2)*. All 3 lesions displayed a dominant mutational signature 1 ascribed to aging. No evidence of clonal selection in the progression of DCIS to IDC-NST was observed in either multifocal DCIS case.

Conclusions: Multifocal DCIS and synchronous IDC-NSTs are genetically heterogeneous. Our data indicate that DCIS can give rise to another DCIS focus via clonal selection, and that genetically unrelated DCIS and IDC-NST may co-exist in the same breast and display similar histologic grade, and ER and HER2 status.

237 Tumor Cell Senescence is Associated with Intraepithelial and Stromal Infiltration of Immune Cells in Breast Cancer and Better Survival in Triple-Negative Breast Cancer

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Disclosures: Min Hui Park: *Employee*, Yeungnam University college of Medicine; Min Chong Kim: None; Jung Eun Choi: None; Young Kyung Bae: None

Background: Senescent tumor cells can modulate their microenvironment by secreting cytokines, chemokines, growth factors, and matrix metalloproteinases. Recent studies reported that SASP has tumor suppressive effects by immune surveillance, but paradoxically, can promote tumor cell growth and epithelial-mesenchymal transition.

Design: To investigate clinicopathologic response to SASP, we examined immunohistochemical expression of senescence markers, p16, high mobility group box-1 (HMGB1), and decoy receptor 2 (Dcr2), in tumor cells of invasive breast carcinoma (IBC) and correlated the results with tumor characteristics including immune cell infiltration and clinical outcome. Immunohistochemistry (IHC) was performed in tissue microarrays of 1518 IBC samples. Positivity for senescence markers was defined as moderate or intense nuclear (p16), cytoplasmic (HMGB1), or cytoplasmic and membranous (Dcr2) staining in ≥5% of tumor cells. Immune cell infiltration was evaluated by stromal tumor-infiltrating lymphocytes (sTILs) density and the number of CD103-positive intratumoral TILs (CD103+ iTILs) per unit area (mm^2).

Results: Positive staining for p16, HMGB1, and Dcr2 was observed in 20.1% (270/1342), 7.2% (96/1337), and 8.6% (120/1402) of cases, respectively. For 1201 cases with IHC results for three senescence markers, 372 (31%) cases were positive for one or more senescent markers (senescence phenotype, SP). SP was significantly associated with an age of < 50 (p=0.023), absence of lymph node (LN) metastasis (p=0.002), absence of lymphovascular invasion (LVI) (p<0.001), histologic grade 3 (p<0.001), high Ki-67 index (p<0.001), and triple-negative breast cancer (TNBC) (p<0.001). IBCs with SP had significantly higher number of CD103+ iTILs (63.9±123.1 *vs* 20.1±72.5, p<0.001) and higher density of sTILs (16±21% vs 7±12%, p<0.001) than those without SP. SP was not associated with overall survival (OS) and disease-free survival (DFS) in all patients, however it predicted better OS (p=0.036) and DFS (p=0.028) in triple-negative subtype of IBC. In multivariate analysis, prognostic significance of SP disappeared. LN status and CD103+ iTILs were independent prognostic factors for OS, and LVI and CD103+ iTILs were for DFS.

Conclusions: In conclusion, SP was frequently observed in TNBC and predicted better survival. Our results support that SP involves in immunologic modulation of tumor microenvironment by inducing stromal and intratumoral infiltration of immune cells.

238 TP53 Mutation Class is Associated with Outcomes and Genomic Landscapes of Endocrine Therapy Resistance

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Disclosures: Edgardo Parrilla Castellar: None; Jennifer Plichta: None

Background: *TP53* is the most commonly mutated gene in breast cancer and mutations in this gene can be pathogenically classified as inactivating, stop-gain (i.e. nonsense, frameshift and splice-site)(class 1, C1) versus missense mutations with dominant-negative activity (class 2, C2). Although *TP53* alterations are associated with high-grade disease, there is limited information on the clinical significance of mutation class.

Design: The Cancer Genome Atlas (https://portal.gdc.cancer.gov) was searched for *TP53* single nucleotide variants and small insertions/deletions in 1,098 primary breast cancers and supplemented with 68 institutional metastatic ER^+/HER^- breast cancer cases. Chi-square test was used for categorical values. Overall survival was estimated and tested using the Kaplan-Meier method and log-rank test.

Results: 370 *TP53* mutations were detected in 358 breast cancer cases, including 136 cases harboring C1 (median age=55 yrs., stage 1-2 N=104, median follow up=29 mo.) and 220 with C2 (medial age=56 yrs., stage 1-2 N=160, median follow up=27 mo.) mutations; 2 cases had mutations in inter-domains and were excluded. C2 mutations prevailed in $ER^+/HER2^-$ (C1, N=30, 28% v. C2, N=76, 72%) and $ER^-/HER2^+$ (C1, N=10, 32% v. C2, N=21, 68%) cases. Class distribution was more similar in $ER^-/HER2^-$ cases (C1, N=64, 46% v. C2, N=75, 54%)(P=0.033). 5-year overall survival rates were 100% and 62% among patients with $ER^+/HER2^-$ breast cancers harboring a C1 (N=64) versus C2 (N=75) mutations, respectively (P=0.022; Fig. 1), with comparable overall disease stage distribution (P=0.457). Overall survival rates were similar between mutation classes in $ER^-/HER2^+$ (P=0.280) and $ER^-/HER2^-$ (P=0.401) cases. Alterations in *ESR1*, *GATA3*, *NF1, EGFR*, *ERBB3* and *KRAS* were more prevalent in metastatic $ER^+/HER2^-$ breast cancer cases harboring C2 mutations compared to cases with C1 mutations (OR=4.250; P=0.008; Fig. 2).

Figure 1 - 238

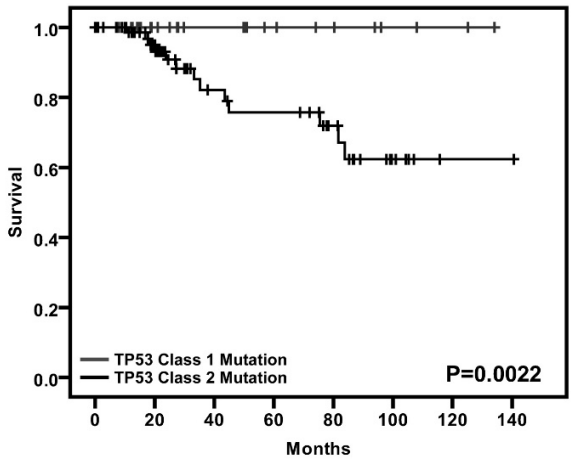
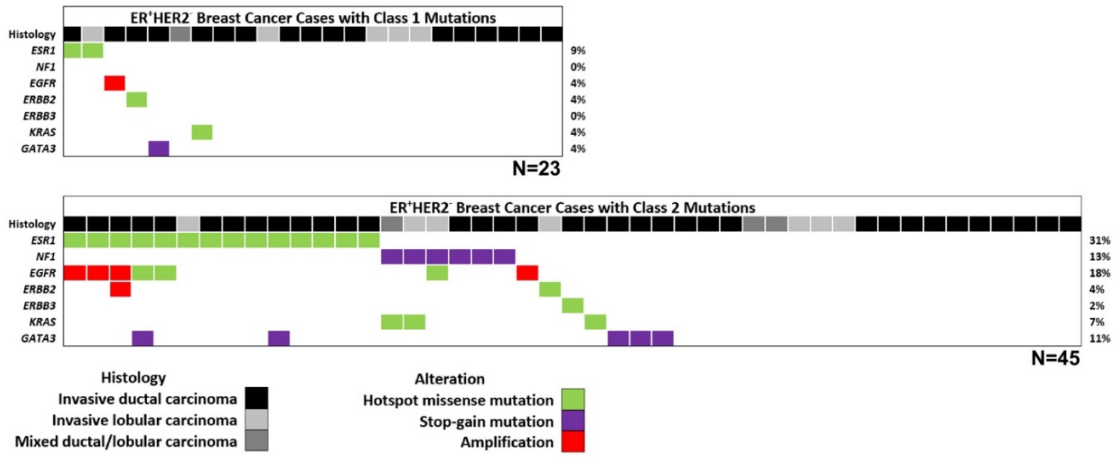


Figure 2 - 238



Conclusions: Patients with ER+/HER2- breast cancers harboring a *TP53* C1 mutation had a more favorable prognosis than those with C2 mutations. Changes associated with endocrine therapy resistance, including mutations in the estrogen machinery, inactivation of *NF1*, and alterations in MAPK signaling (*EGFR*, *ERBB3* and *KRAS*), were 4-fold more common in ER+/HER2- breast cancers with C2 compared to C1 mutations. These results suggest that assessment of *TP53* mutation class may be predictive in ER+/HER2- breast cancer.

239 Patterns of Response to Neoadjuvant Chemotherapy by Breast Cancer Subtype

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Disclosures: Ricardo Pastorello: None; Samantha Grossmith: None; Jungeun Choi: None; Alison Laws: None; Mehra Golshan: None; Elizabeth Mittendorf: None; Stuart Schnitt: None; Tari King: None

Background: The rate of pathologic complete response (pCR) to neoadjuvant chemotherapy (NAC) varies by breast cancer subtype, with the highest rates in triple negative (TNBC) and HER2-positive (HER2+) subtypes. For those not experiencing a pCR, the effect of breast cancer subtype on pattern of residual disease or histologic alterations in the tumor cells/tumor bed has not been studied in detail.

Design: Patients with stage I-III breast cancer treated with NAC and breast conservation (BCT) from 2002-2014 were identified from institutional databases. Histologic sections of the post-treatment surgical specimens were reviewed blinded to tumor subtype. The pattern of tumor regression, treatment-related changes in the tumor cells, and histologic features of the tumor bed were evaluated.

Results: Among 359 patients, slides were available for 269 (75%) cases. Histologic review is complete for 148 (54%) randomly selected cases evaluated in this analysis, including 54 TNBC, 46 HER2+ and 48 estrogen receptor-positive/HER2-negative (ER+/HER2-) cases. An anthracycline and/or taxane based NAC regimen was used in 95.7% of all patients. Among HER2+ cases, 91.3% received neoadjuvant anti-HER2 therapy. pCR (no residual invasive disease in the breast and nodes) was achieved in 44.4%, 43.5%, and 18.8% of TNBC, HER2+, and ER+/HER2- cancers, respectively (p=0.01). For patients with residual disease, there was a significant association between tumor subtype and pattern of response. A relatively circumscribed, concentric pattern of response was seen in 62.5% of TNBCs, 27.3% of HER2+ and 26.7% of ER+/HER2- tumors (p=0.01). Conversely, a scattered pattern of response composed of single cells and small nests in a haphazard pattern was seen in 73.3% of ER+/HER2- tumors and 72.7% of HER2+ cancers but in only 37.5% of TNBC (p=0.01). Stromal tumor-infiltrating lymphocytes (TIL) and several treatment-related changes in the residual invasive cancer cells were significantly more frequent in TNBC (Table 1).

Table 1. Stromal TILs and treatment-related changes in cases with residual invasive disease by tumor subtypes

Features evaluated	Breast cancer subtype			p value
	TNBC	HER2+	ER+/HER2-	
High TILs*	18/30 (60%)	4/24 (16.7%)	9/39 (23.1%)	0.001
Moderate/marked nuclear atypia	28/30 (93.3%)	8/24 (33.3%)	24/39 (61.5%)	<0.001
Cytoplasmic vacuolization/foaminess	28/30 (93.3%)	16/24 (66.7%)	29/39 (74.4%)	0.024
Foamy histiocytes in the tumor bed	11/30 (36.7%)	6/24 (25%)	1/39 (2.6%)	0.001

* High TILs defined as greater than average percentage of TILs (cutoff 17.6%)

Conclusions: Among patients treated with NAC and BCT, the likelihood of pCR, pattern of residual disease and changes in residual tumor cells and/or surrounding stroma varies with breast cancer subtype. The prognostic implications of these observations including both local-regional and distant disease-free outcomes require further study.

240 Local Recurrence of Benign and Borderline Phyllodes Tumors: Analysis of Margin Status and Clinicopathological Features

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Disclosures: Marjorie Perron: None; Camilla Cristando: None; Matthea Machteld Almekinders: None; Zenica Bowser: None; Edi Brogi: None; Melissa Murray: None

Background: Phyllodes tumors (PTs) are rare, biphasic neoplasms, classified into clinically relevant subtypes (benign (BPT), borderline (BLPT) and malignant) based on a combination of histological features. NCCN guidelines recommend wide local excision (EXC) with surgical margins (SM) of ≥1cm for all subtypes of PTs to minimize the risk of local recurrence (LR). However, recent studies have questioned whether the LR rate of BPTs and BLPTs is dependent of SM status. We sought to correlate SM status as well as histologic features with LR in a series of BPTs and BLPTs.

Design: We searched our pathology records for BPT and BLPT treated with EXC between 1990-2015. Clinical information was retrieved from the e-medical records. Three study pathologists reviewed all available slides and classified the PTs according to WHO 2012 criteria. IRB approved the study.

Results: We identified 60 women with 64 PTs including 47 (73.4%) BPTs and 17 (26.6%) BLPTs. Patient median age was 43 years (range 16-77). One woman had 2 PTs, another 4, all in the ipsilateral breast. Mean tumor size was 2.5 ± 1.6 cm (range 0.5-9.5). Twenty-eight (44%) PTs had positive SM at first EXC (21 BPTs, 7 BLPTs), only 11 (7 BPTs, 4 BLPTs) underwent re-excision and all yielded negative SM. Seventeen (25%) PTs had final positive SM (14 BPTs, 3 BLPTs). Four (6.3%) PTs recurred locally: 2 (1 BPT, 1 BLPT) of 17 PTs (11.8%) with positive SM recurred and 2 (1 BPT, 1 BLPT) of 48 PTs (4.2%) with negative SM also recurred. Overall rate of LR was 11.8%

(2/17) for BLPTs and 4.3% (2/47) for BPTs. LR rate was 33% (1/3) in the BLPT group and 7% (1/14) in the BPT group with positive SM. Overall median F/U time was 61 months (range 6-328) and 59 months (range 6-285) for PT with positive SM that did not recur. Three recurrent PTs (1 BPT, 2 BLPTs) showed no grade progression and 1 BPT recurred as a BLPT and had negative SM status. No patient developed metastases. Clinicopathological features such as tumor size and stromal atypia are associated with LR on univariate analysis (*Table 1*). SM status of the index PT did not show statistically significant correlation with LR.

Table 1. Patient's Clinical Characteristics

	All patients (n=60)	Local Recurrence (n=4)	No Local Recurrence (n=56)	p value
Age (mean ± SD) **(median, range)**	43.03 ± 12.03 43 (16-77)	33 ± 11.34 34.5 (16-47)	43.75 ± 11.87 43 (18-77)	0.087
Median follow up time, months (range)	61 (6-328)	22.5 (13-46)	61.5 (6-328)	
Ethnicity				
White	44 (73.3%)	3	41	
Asian	4 (6.7%)	1	3	
Black	5 (8.3%)	0	5	
Hispanic	1 (1.7%)	0	1	
Indian	1 (1.7%)	0	1	
Unknown	5 (8.3%)	0	5	
Histological Features of 64 BPTs and BLPTs				
	All PTs (n=64)	Local Recurrence (n=4)	No Local Recurrence (n=60)	p value
Grade				
Benign PT	47 (73.4%)	2	45	0.2854
Borderline PT	17 (26.6%)	2	15	
Size, cm (mean ± SD) (range)	2.45 ± 1.57 (0.5-9.5)	3.98 ± 2.09 (1.8-6)	2.35 ± 1.5 (0.5-9.5)	0.0452* *t-Test*
Final Margin Status				
Positive	17 (26.6%)	2	15	0.5661
Negative	47 (73.4%)	2	45	
Border				
Circumscribed	45 (70.3%)	2	43	0.3411
Focally infiltrative	11 (17.2%)	1	10	
Infiltrative	8 (12.5%)	1	7	
Stromal Overgrowth				
Absent	62 (96.9%)	4	58	1
Present	2 (3.1%)	0	2	
Mitotic Rate				
<5	52 (81.3%)	2	50	0.1449
5-9	11 (17.2%)	2	9	*t-Test*
>10	1 (1.5%)	0	1	
Stromal Atypia				
Mild	53 (82.8%)	1	52	0.0143*
Moderate	10 (15.7%)	3	7	
Marked	1 (1.5%)	0	1	
Growth pattern				
Intracanalicular	23 (35.9%)	1	22	0.4261
Pericanalicular	28 (43.8%)	2	26	
Both	13 (20.3%)	1	12	
Necrosis				
Focal	63 (98.5%)	4	59	1
Minimal	1 (1.5%)	0	1	
Moderate	0	0	0	
Marked	0	0	0	
Stromal expansion				
Present	22 (34.4%)	2	20	0.6025
Absent	42 (65.6%)	2	40	
Subepithelial increased cellularity				
Present	20 (31.3%)	0	20	0.3002
Absent	44 (68.7%)	4	40	

Increased cellularity within cellular fronds				
Present	8 (12.5%)	0	8	1
Absent	56 (87.5%)	4	52	
Increased cellularity between cellular fronds				
Present	6 (9.4%)	0	6	1
Absent	58 (90.6%)	4	54	
General stromal cellularity				
Mild	38 (59.4%)	1	37	0.2985
Moderate	23 (35.9%)	3	20	
Marked	3 (4.7%)	0	3	

Conclusions: In our cohort, larger tumor size and moderate stromal atypia were associated with LR. LR rate was 7% in the BPT group with positive SM and 33% in the BLPT group with positive SM. These data suggest that a more conservative surgical approach can be considered and re-excision of BPTs with positive SM may not be required in absence of other worrisome clinicopathological features.

241 HER2 Immunohistochemistry in Invasive Micropapillary Breast Carcinoma: Complete Assessment of an Incomplete Pattern

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Disclosures: Marjorie Perron: None; Hannah Wen: None; Matthew Hanna: None; Edi Brogi: None; Dara Ross: None

Background: Invasive micropapillary carcinoma (IMPC) is a rare variant of breast carcinoma, composed of avascular morula-like clusters of tumor surrounded by stromal spaces. This morphology can affect the HER2 immunohistochemical (IHC) staining pattern and the 2013 ASCO/CAP HER2 Guidelines suggest moderate to intense but incomplete (basolateral) staining be considered equivocal. We perform a detailed assessment of HER2 IHC staining patterns in IMPC.

Design: We searched our surgical pathology database for primary IMPC with available HER2 IHC stains diagnosed from 1/2017-9/2019. Clinical details, pathologic diagnoses, hormone receptors (HR) and HER2 IHC and FISH results (if available) were retrieved from e-medical records. H&E and HER2 IHC slides were reviewed by 2 authors to assess the MP component of invasive cancer and HER2 IHC characteristics, including staining intensity, distribution and pattern. The 2018 ASCO/CAP HER2 guidelines were applied.

Results: The cohort consisted of 187 IMPC from 180 women and 2 men with median age 58 years (range 30-95). Procedure was 33% (61) biopsy and 67% (126) excision. Table 1 details histopathology and HER2 IHC patterns. Homogeneous (>90%) MP component was in 40% (75) of cases. Receptor profile was: 75% (141) HR+HER2-, 20% (38) HR+HER2+, 3% (5) HR-HER2+ and 2% (3) HR-HER2-. Of 26 cases with HER2 3+ results, 23% (6) showed complete staining pattern, 46% (12) basolateral and 31% (8) mixed complete and basolateral. HER2 FISH was performed in 36% (68) of cases, including 1 HER2 IHC negative, 66 equivocal and 1 positive (Table 1). FISH was amplified in 26% (17/66) of HER2 IHC equivocal cases, with MP component <60% in 3/17 cases, 60-89% in 5/17 cases, >90% in 9/17 cases. 14/17 (82%) IHC equivocal/FISH amplified cases showed basolateral staining (7 basolateral, 6 complete and basolateral, and 1 basolateral and incomplete) and 3 cases had complete staining (1 complete, 2 complete and incomplete) with weak-moderate intensity in 2 cases, moderate in 14 cases and moderate-strong in 1 case.

Histopathologic features

		Pattern distribution					Mucinous features				Apocrine morphology
Micropapillary component	**All cases (187)**	**Scattered**	**Focal**	**Heterogeneous**	**Contiguous**	**Homogeneous**	**<30 %**	**30-59 %**	**60-89%**	**>90%**	
< 30%	51 (27.3 %)	5	31	5	9	1	3	2	0	0	0
30-59%	28 (15%)	0	2	7	14	5	0	0	1	0	0
60-89%	33 (17.6 %)	0	0	2	6	25	3	1	1	2	3
>90%	75 (40.1 %)	0	0	0	0	75	0	5	4	16	1

HER2 IHC staining pattern

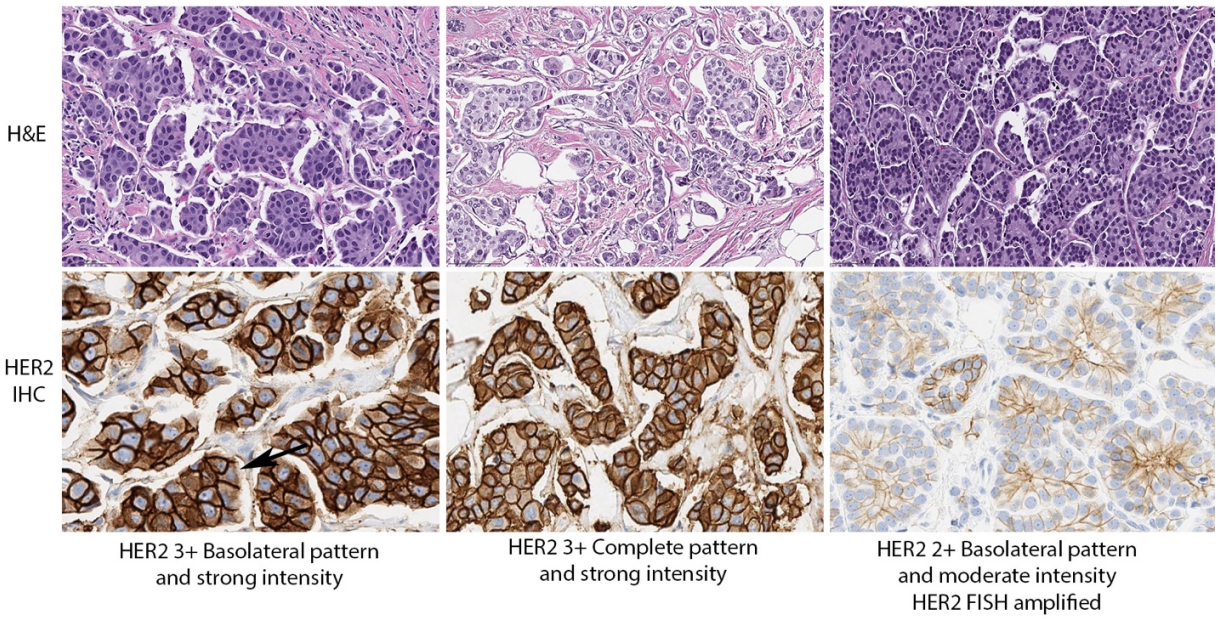
		Staining intensity					Staining distribution		
Micropapillary component	**All cases (187)**	**Weak**	**Weak to moderate**	**Moderate**	**Moderate to strong**	**Strong**	**Focal**	**Heterogeneous**	**Homogenous**
Absence	25 (13%)	0	0	0	0	0	0	0	0
Incomplete	44 (24%)	44	0	0	0	0	16	26	2
Complete	11 (6%)	2	0	3	0	6	0	3	8
Basolateral	52 (28%)	16	7	15	2	12	8	20	24
Complete and incomplete	8 (4%)	1	4	3	0	0	0	8	0
Complete and basolateral	26 (14%)	1	5	12	0	8	1	14	11
Incomplete and basolateral	19 (10%)	14	2	3	0	0	1	16	0
Complete, incomplete and basolateral	2 (1%)	0	2	0	0	0	1	1	0

HER2 IHC and FISH results

HER2 IHC reviewed		**FISH testing results**						
HER2 IHC group	**All cases (187)**	**All cases (68)**	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**IND**
Negative 0	30 (16%)	0	0	0	0	0	0	0
Negative 0 to 1+	2 (1%)	0	0	0	0	0	0	0
Negative 1+	63 (34%)	1	0	0	0	0	1 (100%)	0
Equivocal 1+ to 2+	11 (6%)	11	1 (9%)	0	0	2 (18%)	7 (64%)	1 (9%)
Equivocal 2+	55 (29%)	55	14 (25%)	0	2 (4%)	13 (24%)	25 (45%)	1 (2%)
Positive 3+	26 (14%)	1	1 (100%)	0	0	0	0	0

Abbreviations: IND (Indeterminate); IHC (Immunohistochemistry)

Figure 1 - 241



Conclusions: Our results show that staining pattern and intensity are key features in the assessment of HER2 IHC in IMPC. The most frequent staining pattern in this large cohort was basolateral, seen in 53% of cases, including 77% HER2 IHC positive and 82% HER2 IHC equivocal/FISH positive. All HER2 IHC 3+ cases showed strong intensity. If a basolateral pattern and weak to moderate staining is observed in IMPC, alternative testing should be performed to confirm the HER2 status.

242 Early Results of PD-L1 Staining and Atezolizumab (atezo) Treatment (Tx) in Patients with Metastatic Triple Negative Breast Carcinoma (TNBC)

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Disclosures: Jessica Peters: None; Ira Bleiweiss: None; Paul Zhang: None; Kimberly Dumoff: None; Anupma Nayak: None

Background: The FDA approval of atezo for clinical use in TNBCs following IMpassion130 trial has placed pathologists in a challenging role as they are often required to optimize and validate companion dx assays in labs and gain proficiency in reading these test results. At present, there is confusion amongst pathologists and treating oncologists regarding the type of tissue (primary vs. metastasis) to be tested, type of antibody to be used and type of scoring methods to apply. Herein, we present our experience with PD-L1 staining in pts with metastatic TNBCs.

Design: 48 tumors from 40 pts. underwent PD-L1 staining between March - July 2019 using SP142 antibody. Factors of interest included- tumor tested (primary or metastatic), % of tumor infiltrating immune cells (IC), patient follow up, and Tx side effects, if any. PD-L1 scoring was based on PD-L1 expressing ICs as % of tumor area: IC1 ≥1% as positive while IC0 <1% as negative.

Results: Of 48 tumors, 28 were primary and 20 metastatic (6 axillary LNs, 5 liver, 3 lung, 2 brain, 2 skin, 1 abdominal wall & 1 supraclavicular soft tissue). Six were tested on both primary and metastatic tumors and 2 on 2 primaries. 16/48 tumors were negative, 2 indeterminate because of lack of ICs, and 30 positives (score 1 - 30%). Of the 6 pts. with both primary and metastatic tumors tested, PDL-1 results were concordant in all, with both sites being positive. 22 pts (55%) had positive IC score and were thus eligible for Tx, of which 5 were LFU, 9 received atezo, 4 were recommended for atezo but have not yet started, 1 pt. was deemed ineligible for Tx and 3 pts. expired without any Tx. 2/9 pts. expired within 1 month of Tx. One of the 2 deceased pts. developed PD-L1 pneumonitis; however, the brain metastasis had decreased in size. Of the remaining 7 pts. undergoing Tx, 1 developed a rash but showed shrinkage of liver nodules, 1 reported fatigue and arthralgias, 1 had an enlarged supraclavicular LN but improvement of symptoms elsewhere, 1 had no improvement in carcinomatosis and presented with symptoms of pulmonary embolism. The remaining 3 pts did not report any AEs.

Conclusions: In this study, 55% of pts. with metastatic TNBCs had PD-L1 positivity by current criteria in either primary carcinoma, metastasis, or both. When feasible, staining of primary tumor is preferable because of the relatively small size of most metastatic tissue samples, the paucity of ICs in most metastases, and the inability to perform testing on bone metastases or cytology specimens.

243 Characteristics of Screen-Detected Breast Cancer with and without 3D Tomography

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Disclosures: Jessica Peters: None; Ira Bleiweiss: None; Anupma Nayak: None

Background: Mammography is currently being utilized as a screening tool to detect early breast cancers using low dose x-rays. Digital mammography allows for higher quality images with less radiation. Breast tomosynthesis (3D mammography)/digital breast tomosynthesis takes multiple images of the breast from different angles and then creates a 3D image. We investigated if there is a difference between breast cancers detected by 3-D tomography.

Design: 200 breast cancer patients were selected, 100 who were screened via tomography (T) and one hundred who were not (NT) from September 2010 to April 2013. The patients ranged in age from 34 to 78 (T group) and 36 to 79 (NT group). We noted histologic subtype and grade, radiologic size, radiologic presentation (including mass, calcifications, architectural distortion and density), whether the lesion was associated with ductal carcinoma in situ (DCIS), lobular carcinoma in situ (LCIS) or other lesions (including atypical duct hyperplasia, radial scar, intraductal papilloma, mucocele-like lesion and fibroadenoma) and predictive marker status (estrogen receptor (ER), progesterone receptor (PR) and Her2).

Results: Approx. 2/3 thirds of the patients (NT group) had invasive carcinoma (IC) (54 ductal, 4 lobular, 3 mixed ductal and lobular features, 3 with separate ductal and DCIS, 1 invasive mammary, undesignated, 1 microinvasive), and 1/3 had DCIS. Similarly approx. 2/3 thirds of the T group patients had invasive carcinoma (45 ductal, 7 lobular, 2 invasive mammary, unspecified, 3 mixed ductal and lobular features, 2 with separate ductal and DCIS, 2 microinvasive), and 1/3 had DCIS. For NT, roughly 50% of the IC presented as a mass, 10% with calcifications, 2% with distortion, 1% with a lesion and the remainder are unknown. The smallest size of IC detected was 4 mm. The grade distribution of invasive duct carcinomas in both groups was nearly identical (NT-21% grade I, 62% grade II, 17% grade III) versus (T-25% grade I, 57% grade II, 18% grade III). Average size of invasive tumors, predictive marker status, and type of DCIS showed similar distributions between the two groups.

Conclusions: No significant differences can be identified between NT and T screened carcinomas of the breast although further studies with larger patient cohorts are necessary.

244 Analysis of Real-World PD-L1 Testing and PD-L1 IHC 28-8 and 22C3 Assay Concordance in Patients with Breast Cancer

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Disclosures: Emily Prince: *Employee*, Bristol-Myers Squibb; *Employee*, Bristol-Myers Squibb; James Pratt: *Employee*, Bristol-Myers Squibb; *Employee*, Bristol-Myers Squibb; James Novotny: *Employee*, Bristol-Myers Squibb; *Employee*, Bristol-Myers Squibb; *Stock Ownership*, Merck; *Stock Ownership*, AstraZeneca; *Stock Ownership*, GlaxoSmithKline; Vladislav Chizhevsky: *Employee*, Neogenomics Laboratories Inc; *Employee*, Neogenomics Laboratories Inc; *Stock Ownership*, Seattle Genetics; Josette William Ragheb: *Employee*, Neogenomics Laboratories Inc; David Huron: *Employee*, Bristol-Myers Squibb; *Employee*, Bristol-Myers Squibb

Background: Programmed death-1/programmed death ligand 1 (PD-1/PD-L1) inhibitors have been approved for use in a range of tumor types. PD-L1 expression, as assessed using FDA-approved immunohistochemistry (IHC) diagnostic assays such as the Dako PD-L1 IHC 28-8 and 22C3 pharmDx assays or the Ventana PD-L1 (SP142) assay, is associated with improved outcomes following PD-1/PD-L1 inhibitor treatment in some tumor types. Atezolizumab, in combination with nab-paclitaxel, was approved for the treatment of patients with PD-L1+ advanced triple-negative breast cancer in Mar 2019, with SP142 approved as a companion diagnostic assay. Here, we investigated the prevalence of PD-L1 expression, test utilization, and analytical concordance in real-world breast cancer (BC) samples.

Design: NeoGenomics Laboratories Inc (Fort Myers, FL, USA), a US national reference laboratory, provided results for PD-L1 tests performed between Oct 2015 and Dec 2018. Clinical characteristics of patients were provided by Symphony Healthcare Solutions and matched to test results using unique identifiers. PD-L1 expression was determined by trained pathologists using the 28-8 or 22C3 assays (Agilent Technologies), or the SP142 assay (Ventana Medical Systems), according to the manufacturers' protocols at the time. PD-L1 expression was reported as the percentage of tumor cells (TCs) with PD-L1 staining for the 28-8 and 22C3 assays only. Statistical analysis was performed by BioStat Solutions Inc.

Results: 623 PD-L1 tests were performed on samples from 577 patients with BC. The volume of PD-L1 tests on BC samples increased ~20-fold over the period evaluated. Mean turnaround time (TAT) was <5 days. Prevalence of PD-L1 expression on TCs in patients with a 28-8 or 22C3 test result (n=549) is shown in the table. Concordance between the 28-8 and 22C3 assays in matched samples from 26 patients with BC was high (Kendall's tau=0.997 [95% CI, 0.889–1.000]) (Figure). Overall (OPA), positive, and negative percentage agreement between the 28-8 and 22C3 assays were 100% (Cohen's kappa 1.00) at all cutoffs evaluated. Assessment of PD-L1 expression by biopsy location is ongoing.

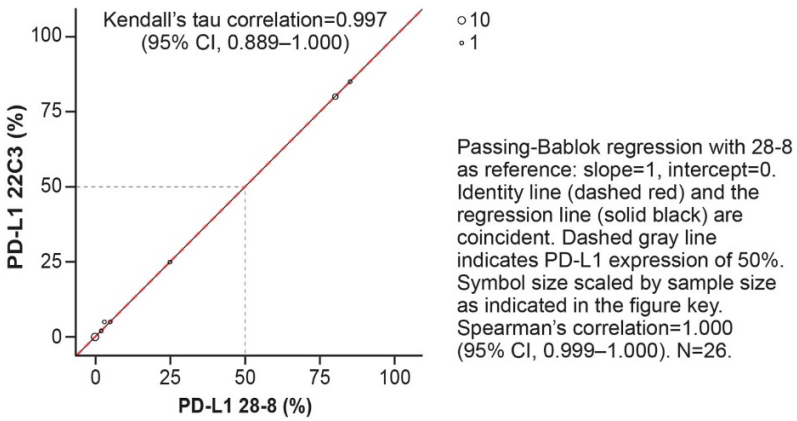
Table. Prevalence of PD-L1 expression in BC

PD-L1 expression, % TCs	Patients, n (%) (n=549)[a]
0%	355 (65)
1%–24%	127 (23)
25%–49%	24 (4)
50%–100%	43 (8)

[a]Patients with a single test result or ≥2 identical test results for the 28-8 or 22C3 assays.

Figure 1 - 244

Figure. Agreement between the 28-8 and 22C3 assays in patients with BC



Conclusions: Mean PD-L1 test TAT for BC samples remained <5 days despite a large increase in test volume. Tumor PD-L1 expression was ≥1% in 35% of samples. Despite a small sample size, analytical concordance between the 28-8 and 22C3 assays in matched samples was high (OPA=100%). These data provide real-world context for PD-L1 testing in BC.

245 Receptor Conversion in Metastatic Breast Cancer: Analysis of 241 Cases

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Disclosures: Morad Qarmali: None; Rong Chen: None; Gene Siegal: None; Shi Wei: None

Background: Breast cancer (BC) mortality is the final sequelae of distant relapse in the majority of such cases. Estrogen receptor (ER), progesterone receptor (PR) and HER2 status provide clinical utility in guiding therapeutic decision-making in metastatic BC. Endocrine or HER2-targeted therapies are mainly directed at the receptor status of the primary tumors. However, increasing data have shown substantial differences between the receptor profiles of primary BCs and their paired metastases. In this study, we sought to assess the frequency of receptor conversion in a large single center cohort.

Design: The receptor statuses of primary BCs and their paired metastases diagnosed at the authors' institution between 01/2000 and 08/2019 were retrospectively collected following IRB approval. Those from regional lymph nodes were excluded. A total of 241, 222 and 214 paired cases were available in the study period for ER, PR and HER2 analyses, respectively. Semiquantitative analyses of ER and PR using H-score were also performed, when available.

Results: The highest conversion rate was seen for PR (41.4%), including 72 (32.4%) positive to negative (+/-) and 20 (9%) negative to positive (-/+). The mean PR H-scores of the primary BCs and their paired metastases were 84.4 and 36.5, respectively (*P*<.0001). The conversion rate for ER was 17.4%, including 35 (14.5%) +/- and 7 (2.9%) -/+, while the mean H-scores of the primary and metastatic BCs were 190.7 and 155.4, respectively (*P*=.01). HER2 conversion rate was 11.7%, including 14 (6.5%) +/- and 11 (5.2%) -/+. The discordant rate was significantly higher for PR when compared to ER and HER2 (both *P*<.0001), while no significant difference was found for the latter two. When the sites of metastasis were analyzed separately, PR conversion rate was significantly higher in bone relapse (56.5%) when compared to those metastasized to the liver (*P*=.046) or lung/pleura (*P*=.02), but not brain. No significant conversation rate for the sites of relapse was found for ER or HER2.

Conclusions: Receptor discordance is a frequent event in the course of BC progression, and can be a +/- or -/+ conversion. Over 40% of patients receptor status was converted in metastatic tumors. The findings may reflect tumor heterogeneity, sampling or treatment effect, and may indicate alteration in tumor biology. Thus, repeat biomarker studies are warranted in making appropriate treatment plans in the era of precision medicine.

246 Immuno-Oncology Biomarker Expression in Breast Cancer by mRNA-Nanostring and Protein-IHC Analysis

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Disclosures: Kimmie Rabe: None; Todd Knutson: None; Ryan Shanley: None; Colleen Forster: None; Molly Klein: None; Andrew Nelson: None

Background: Checkpoint therapy shows promise for breast cancer treatment; however, uncertainty exists regarding the best immuno-oncology (IO) biomarkers for personalizing breast cancer treatment. In this work, we characterize breast cancer immune biomarker expression using mRNA expression and protein immunohistochemistry (IHC).

Design: We analyzed tumor samples from a retrospective cohort of 120 breast cancer patients (49% ER+, 32% HER2+, and 19% triple negative), with recorded clinicopathologic (CP) variables and 5 year survival outcomes. Tumor samples were analyzed by a custom Nanostring gene expression assay of immune/stromal genes and the PAM50 classifier. Immunohistochemistry on whole tumor sections was performed for: PDL1, PD1, CTLA4, CD8, and CD68. Tumor infiltrating lymphocytes (TILs) were assessed on H&E. Statistics were performed with logistic or proportional odds regression, ANOVA, or Fisher's exact test.

Results: Increased PD1 and CTLA4 mRNA levels were associated with lymph node (LN) positive status (p=0.01, 0.02 respectively) and CTLA4 was associated with higher Nottingham grade (p<0.001). CTLA4 expression varied by PAM50 subtype, and was highest in basal and HER2-enriched groups (p=0.003). PDL1 mRNA expression was not significantly associated with CP variables. Unsupervised clustering using all immune/stromal genes segmented cases strongly based on grade, LN status, and PAM50 subtype (Fig.1). Preliminary IHC scoring for PDL1, PD1, and CTLA4 (Table 1) demonstrated expression more frequently in immune cells than in tumor cells. IHC scoring and mRNA expression were not strongly correlated. In contrast to mRNA data, PDL1 and PD1 expression by IHC were associated with positive LN status (both p=0.02); but CTLA4 expression by IHC showed no association. TILs ranged from 1-80, with a mean of 17. The mean proportion of CD8+ lymphocytes was 35%.

Table 1: CTLA4, PD1, and PDL1 IHC Results

Biomarker	Proportion of total cases positive (>1% IC+)	Mean % IC+ per case	Range % IC+ per case
CTLA4	83%	7%	1-25%
PD1	40%	4%	1-25%
PDL1	34%	4%	1-30%

IC=immune cells

Figure 1 - 246

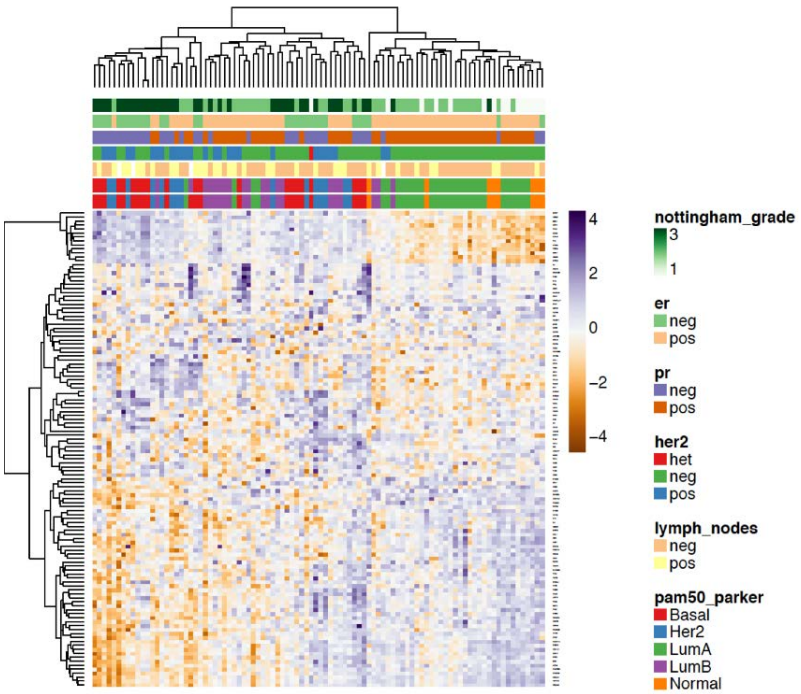

Figure 1: Unsupervised hierarchical clustering using 120 immune/stromal associated genes. Clinicopathologic variables are indicated across the top banners as defined at right. The PAM50 algorithm was run twice independently (two bottom tracks) with minor case reassignment.

Conclusions: Our data suggest caution is needed in the comparison of mRNA to IHC data for IO biomarkers in breast cancer samples. Nonetheless, both techniques identify significant associations of IO biomarkers with LN metastasis. After completing IHC scoring for the entire cohort, we will further explore correlations between individual IO biomarkers, and further test associations with all CP variables.

247 Prognosis of Triple Negative Breast Cancers after Adjuvant Chemotherapy Classified by Intrinsic Subtype and Immunity Score

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Disclosures: Xinyu Ren: None; Songjie Shen: None; Xi Cao: None; Qiang Sun: None

Background: Adjuvant chemotherapy is still the major treatment for triple negative breast cancers (TNBC) after surgical resection. However, the prognosis is quiet different among TNBC patients receiving similar chemotherapeutical regimens.

Design: To observe how intrinsic subtypes and immune status within the tumor affect the prognosis of TNBCs after adjuvant chemotherapies. A total of 111 retrospective tumor samples of TNBC were studied including 47 cases of relapse and 64 cases of non-relapses with a median follow-up time of 92 months. RNA was extracted from FFPE tumor samples and target gene expression profiles were tested by Next generation of sequencing (NGS). Intrinsic subtypes were analyzed using PAM50 and a group of 17 immune genes was used for immunity evaluation within the tumors.

Results: PAM50 analysis showed all intrinsic subtypes existed in TNBCs, including luminal A（13，12%）, luminal B（1, 1%）, HER2-enriched（11, 10%） and Basal-like（86，77%）. The intrinsic subtypes showed a significant impact on prognosis for relapse-free survival in the TNBC patients. While Basal-like showed an expected outcome as reported before, HER2-eriched and Lumina A both had

worse outcome than Basal-like. Among the patients with Luminal A subtype, 10 of 13 were ER or/and PR positive detected at RNA level, and risk of recurrence (ROR) showed 5 were in low risk and 8 were in medium risk. For HER2-enriched patients, 3 of 11 were HER-2 positive at RNA level. Immunity score was calculated with expression of 17 immune-related genes and divided patients into immune-strong and immune-weak groups. Immunity score had significant impact on prognosis only in late stage patients (IIB and above) and was significantly correlated with the amount of tumor infiltrated lymphocytes.

Conclusions: Intrinsic subtype-dependent impact on prognosis of TNBC patients after adjuvant chemotherapies was found in current study. Patients with intrinsic subtypes other than Basal-like may need different therapeutic strategies, such as endocrine or target therapies, to improve their prognosis. Immunity score is a good predictor for prognosis of TNBC in late stage. A larger scale of perspective study is needed to confirm findings in current study.

248 Metastatic Breast Carcinoma in Women, Body Mass Index, and Survival

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Disclosures: Cynthia Reyes Barron: None; Maricarmen Planas-Silva: None; David Hicks: None; Bradley Turner: None

Background: Breast cancer is the most prevalent cancer in women worldwide. Women with distant metastases have poor outcomes with frequent development of resistance to available hormonal and chemotherapeutic agents and worse overall survival. According to the Centers for Disease Control, the national average body mass index (BMI) for adult women in the U.S. in 2015 was 28.6 kg/m^2, which is considered overweight. Conflicting evidence exists regarding the association of BMI with disease progression free survival and overall survival in patients with metastatic breast cancer.

Design: A single institution retrospective study of women with metastatic breast cancer was conducted. Pathology reports and medical records from 2011 to 2015 were searched to identify cases with biopsy proven breast metastases to any distant organ site. Several factors were considered including breast cancer histologic type and stage at diagnosis, estrogen receptor (ER)status , progesterone receptor status, HER2-neu amplification status, the type of metastatic disease, metastatic site, patient age, ethnicity, BMI (kg/m^2) at the time of metastases, and outcome to include survival after metastases.

Results: 127 cases of metastatic breast cancer were identified. 118 of these had BMI data at the time of the first metastatic diagnosis. Ages ranged from 32 to 91 years of age (mean 63.4). BMI ranged from 17.4 kg/m^2 to 52.9 kg/m^2 (mean 29.3). 73% of patients were overweight or obese. More patients were obese in all categories with metastatic disease (liver, bone only, other, ER+, and triple negative) except HER2+, where most patients were overweight. Only one patient was underweight. Outcome data was available for 111 cases. 74 (67%) patients were deceased at the time of the study with an average survival of 863.4 days after first metastasis diagnosis. 22 patients survived greater than 2000 days (Table).

Category	All[1]			Liver +/- other site mets			Bone only mets			Other (no bone and no liver mets)			ER+			HER2+			Triple Negative		
BMI kg/m^2	N (%)	Mean survival in days[1]	Survivors >2000 days (%)[3]	N (%)	Mean survival in days[2]	Survivors >2000 days (%)[3]	N (%)	Mean survival in days[2]	Survivors >2000 days (%)[3]	N (%)	Mean survival in days[2]	Survivors >2000 days (%)[3]	N (%)	Mean survival in days[2]	Survivors >2000 days (%)[3]	N (%)	Mean survival in days[2]	Survivors >2000 days (%)[3]	N (%)	Mean survival in days[2]	Survivors >2000 days (%)[3]
Underweight <18.5	1 (0.8)	40	0 (0)	0 (0)	N/A	N/A	0 (0)	N/A	N/A	1 (6.3)	40	0 (0)	0 (0)	N/A	N/A	0 (0)	N/A	N/A	1 (6.3)	40	0(0)
Normal 18.5-24.9	31 (26.3)	930.8	7 (22.6)	16 (33.3)	619.5	4 (25)	5 (16.7)	419.3	1 (20)	3 (18.7)	1325	0 (0)	23 (29.1)	993.8	6 (26.1)	2 (13.3)	911.5	0 (0)	3 (18.7)	599.3	0(0)
Overweight 25.0-29.9	34 (28.8)	785.2	7 (20.6)	15 (31.3)	734.3	1 (6.7)	8 (26.7)	N/A	3 (37.5)	5 (31.3)	413	1 (25)	21 (26.6)	926.3	6 (25)	7 (46.7)	1259.6	3 (42.9)	5 (31.3)	457.2	0(0)
Obese ≥30	52 (44.1)	899.3	8 (15.4)	17 (35.4)	841.7	2 (11.7)	17 (56.6)	539.7	2(11.8)	7 (43.7)	645.5	2 (28.6)	35 (44.3)	1078	6 (17.1)	6 (40)	1200.7	2 (28.6)	7 (43.7)	408.8	1 (14.3)
Total cases	118			48			30			16			79			15			16		
Avg BMI	29.3			28.8			31.4			28.2			29.5			28.9			27.7		

[1] Includes all metastatic disease. Bone metastases without liver metastases (but with metastases to other sites) are not included as a separate category in this table due to limited numbers

[2] Mean survival from time of diagnosis of metastatic disease in patients that were deceased at time of study

[3] Number of cases with known follow-up with greater than 2,000 days survival after diagnosis of metastatic disease and % in BMI category

N/A not applicable

Figure 1 - 248

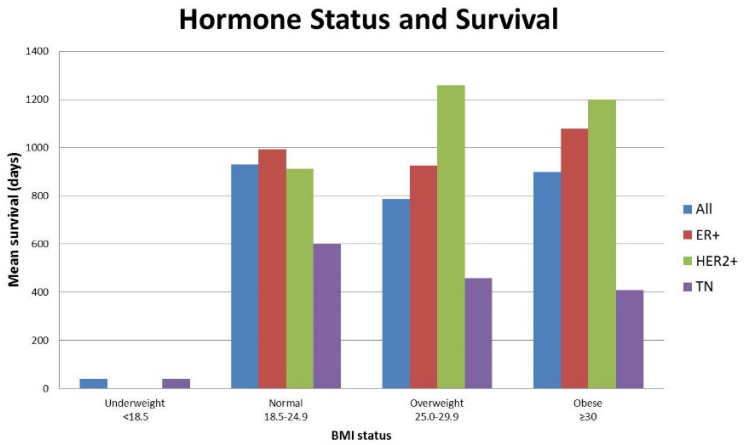
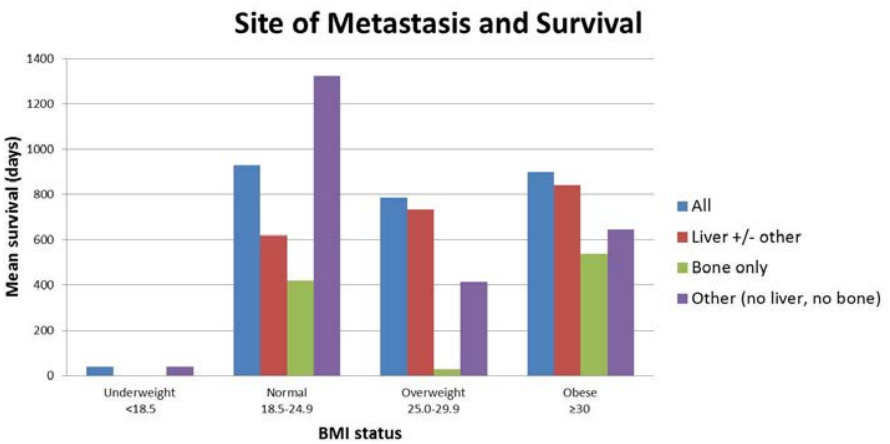


Figure 2 - 248



Conclusions: Obesity is a well-known cause of co-morbidities, and has been suggested to negatively impact the prognosis in breast cancer. In our study population, BMI did not significantly impact the site of metastasis, and there was no significant correlation between overall mean survival and BMI; however, there was a noticeable trend for *decreased* survival with increasing BMI in triple negative patients (Fig1), and a noticeable trend for *increased* survival with increasing BMI in patients with liver metastases (Fig 2). Further investigation into tumor hormonal status, site of metastatic disease, and BMI are warranted in larger cohorts.

249 NR4A3 Expression is Consistently Absent in Acinic Cell Carcinomas of the Breast: A Potential Nosologic Shift

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Disclosures: Edward Richardson: None; Fresia Pareja: None; Jorge Reis-Filho: None; Jason Hornick: *Consultant*, Eli Lilly; *Consultant*, Epizyme; Vickie Jo: *Employee*, Merck and Co; Stuart Schnitt: None

Background: Several carcinomas of the breast are histologically and genetically identical to their salivary gland counterparts (e.g., adenoid cystic carcinoma: *MYB-NFIB*; secretory carcinoma: *ETV6-NTRK3*; mucoepidermoid carcinoma: *CRTC1-MAML2*). Acinic cell carcinoma (AciCC) is yet another tumor that occurs in both the breast and salivary glands. Recently, a recurrent genomic rearrangement, t(4;9)(q13;q31), was identified in salivary AciCC which results in constitutive upregulation of the nuclear transcription factor NR4A3 that can be detected by immunohistochemistry (IHC). Nuclear NR4A3 staining has been found to be a sensitive and specific marker of AciCC of the salivary gland, but its expression in AciCC of the breast has not yet been studied.

Design: To address this issue, NR4A3 IHC was performed on whole-tissue sections of 13 cases of AciCC of the breast from 11 unique patients. Nuclear staining was considered a positive result.

Results: The median patient age was 48 years (range, 32-74) and the median tumor size was 2.5 cm (range, 0.6-10.5). 8 cases had axillary lymph nodes sampled; one patient had 10/17 positive lymph nodes, one patient had isolated tumor cells in one lymph node, and the remainder were node-negative. 6 tumors showed mixed histologic patterns, most commonly AciCC admixed with invasive ductal carcinoma of no special type (4 cases) followed by AciCC admixed with metaplastic carcinoma (2 cases). All tumors showed the characteristic histologic features (haphazardly distributed small glands and solid nests with eosinophilic luminal secretions in the glands, cells with finely to coarsely granular cytoplasm) and immunophenotype (e.g. positive for S100, lysozyme, and alpha-1-antichymotrypsin, and negative for ER, PR, and HER2) of AciCC. For tumors with mixed histologic patterns, the areas of AciCC were analyzed for NR4A3 staining. All 13 breast AciCC were negative for NR4A3.

Conclusions: We report that breast AciCC are consistently negative for NR4A3 by IHC, despite this marker being expressed in most salivary AciCC. Despite the morphologic similarities, our findings suggest that the molecular underpinnings of salivary gland and breast AciCC are different. This study complements prior work and provides further evidence that a tumor type that occurs in both the breast and salivary glands may have different underlying genetic abnormalities in each organ. Further studies are needed to determine the molecular drivers of AciCC in the breast.

250 Results of a Decade of MRI-Guided Core Needle Breast Biopsies: A Single Institution's Experience

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Disclosures: Kara Roncin: None; Hannah Gilmore: None; Aparna Harbhajanka: None

Background: With the advancement of screening practices along with molecular assays that require increasingly smaller amounts of tissue, there is a drive to improve the patient experience when evaluating enhancing and non-enhancing mass lesions of the breast by utilizing less invasive methods. Widespread adoption of MRI guided vacuum-assisted core needle biopsies is a modality suited to evaluating non-palpable breast lesions discovered on routine screening in order to provide earlier detection of clinically significant lesions.

Design: A retrospective natural language search for MRI guided breast biopsies reviewed within a single institutional database between 01/01/2010 and 06/14/2019 yielded 329 cases and 414 unique specimens from 322 patients (age range: 21-79 years old). The final diagnosis for each case was stratified into the following diagnostic findings: invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), ductal carcinoma in situ (DCIS), atypical ductal hyperplasia (ADH), lobular carcinoma in situ (LCIS), atypical lobular hyperplasia (ALH), intraductal papilloma (IP), treatment effect (RX), surgical/biopsy effect (SURG), flat epithelial atypia (FEA), columnar cell change (CCC), fibroadenoma/fibroadenomatous change (FA), and apocrine metaplasia (APO). All remaining entities were considered benign. The results were further categorized into the following: invasive (IDC, ILC), DCIS, high-risk atypical (ADH), non-high-risk atypical (LCIS, ALH, IP, FEA), and benign (RX, SURG, CCC, FA, APO). Chi-square analysis was performed.

Results: Of the 414 MRI guided biopsies evaluated, 266 (64.3%) were benign, 49 (11.8%) were non-high risk atypical, 22 (5.3%) were ADH, 36 (8.7%) were DCIS, and 41 (9.9%) showed invasive carcinoma. The most common diagnosis was apocrine metaplasia (124; 30.0%) (Table 1). Benign diagnoses were most frequently identified in women, 30-39 years old (25/30; 83.3%). Malignant diagnoses (invasive carcinoma or DCIS) were most frequently identified in women, 60-69 years old (20/58; 34.4%) (Figure 1). The majority of cases were from Caucasian women, 40-49 years old (95/322; 29.5%) (Figure 2). There was no statistical significance in women from other ethnicities.

TABLE 1: Patient Characteristics by Age and Ethnic Background at Time of Initial MRI-Guided Vacuum Assisted Core Needle Biopsy. A total of 322 patients, divided by age and ethnicity, as declared by the patient. Ductal Carcinoma in Situ (DCIS), Atypical Ductal Hyperplasia (ADH), White (W), Black (B), Asian (A), and other/unknown (U).

Age (Years)	Invasive Carcinoma	DCIS	High-Risk Epithelial Atypia (ADH)	Non-High Risk Epithelial Atypia	Benign	Total
ALL (21-79)	11.5% (37) W: 89.2% (33/37) B: 8.1% (3/37) A: 2.7% (1/37) U: 0%	9.6% (31) W: 77.4% (24/31) B: 22.6% (7/31) A: 0% U: 0%	6.2% (20) W: 95.0% (19/20) B: 5.0% (1/20) A: 0% U: 0%	11.5% (37) W: 86.5% (32/37) B: 5.4% (2/37) A: 2.7% (1/37) U: 5.4% (2/37)	61.2% (197) W: 84.8% (167/197) B: 10.2% (20/197) A: 1.5% (3/197) U: 3.6% (7/197)	100% (322) W: 85.4% (275/322) B: 10.3% (33/322) A: 1.6% (5/322) U: 2.8% (9/322)
20-29	6.3% (1/16) W: 8.3% (1/12) B: 0% A: 0% U: 0%	6.3% (1/16) W: 0% B: 33.0% (1/3) A: 0% U: 0%	0%	6.3% (1/16) W: 0% B: 0% A: 0% U: 100% (1/1)	81.3% (13/16) W: 91.7% (11/12) B: 66.7% (2/3) A: 0% U: 0%	5.0% (16/322) W: 75.0% (12/16) B: 18.8% (3/16) A: 0% U: 6.3% (1/16)
30-39	0%	3.3% (1/30) W: 4.6% (1/22) B: 0% A: 0% U: 0%	0%	13.3% (4/30) W: 4.6% (1/22) B: 25.0% (1/4) A: 50% (1/2) U: 50% (1/2)	83.3% (25/30) W: 90.9% (20/22) B: 75.0% (3/4) A: 50% (1/2) U: 50% (1/2)	9.3% (30/322) W: 73.3% (22/30) B: 13.3% (4/30) A: 6.7% (2/30) U: 6.7% (2/30)
40-49	6.5% (7/108) W: 7.4% (7/95) B: 0% A: 0% U: 0%	11.1% (12/108) W: 12.6% (12/95) B: 0% A: 0% U: 0%	11.1% (12/108) W: 11.6% (11/95) B: 14.3% (1/7) A: 0% U: 0%	8.3% (9/108) W: 9.5% (9/95) B: 0% A: 0% U: 0%	63.0% (68/108) W: 59.0% (56/95) B: 85.7% (6/7) A: 100% (2/2) U: 100% (4/4)	33.5% (108/322) W: 88.0% (95/108) B: 6.5% (7/108) A: 1.9% (2/108) U: 3.7% (4/108)
50-59	18.6% (16/86) W: 18.2% (14/77) B: 14.3% (1/7) A: 100% (1/1) U: 0%	4.6% (4/86) W: 5.2% (4/77) B: 0% A: 0% U: 0%	3.5% (3/86) W: 3.9% (3/77) B: 0% A: 0% U: 0%	16.3% (14/86) W: 16.9% (13/77) B: 14.3% (1/7) A: 0% U: 0%	57.0% (49/86) W: 55.8% (43/77) B: 71.4% (5/7) A: 0% U: 100% (1/1)	26.7% (86/322) W: 89.5% (77/86) B: 8.1% (7/86) A: 1.2% (1/86) U: 1.2% (1/86)
60-69	17.2% (10/58) W: 20.41% (10/49) B: 0% A: 0% U: 0%	17.2% (10/58) W: 10.2% (5/49) B: 62.5% (5/8) A: 0% U: 0%	5.2% (3/58) W: 6.1% (3/49) B: 0% A: 0% U: 0%	10.3% (6/58) W: 12.2% (6/49) B: 0% A: 0% U: 0%	50.0% (29/58) W: 51.0% (25/49) B: 37.5% (3/8) A: 0% U: 100% (1/1)	18.0% (58/322) W: 84.5% (49/58) B: 13.8% (8/58) A: 0% U: 1.7% (1/58)
70-79	12.5% (3/24) W: 5.0% (1/20) B: 50% (2/4) A: 0% U: 0%	12.5% (3/24) W: 10.0% (2/20) B: 25.0% (1/4) A: 0% U: 0%	8.3% (2/24) W: 10.0% (2/20) B: 0% A: 0% U: 0%	12.5% (3/24) W: 15.0% (3/20) B: 0% A: 0% U: 0%	54.2% (13/24) W: 60.0% (12/20) B: 25.0% (1/4) A: 0% U: 0%	7.4% (24/322) W: 83.3% (20/24) B: 16.7% (4/24) A: 0% U: 0%

Figure 1 - 250

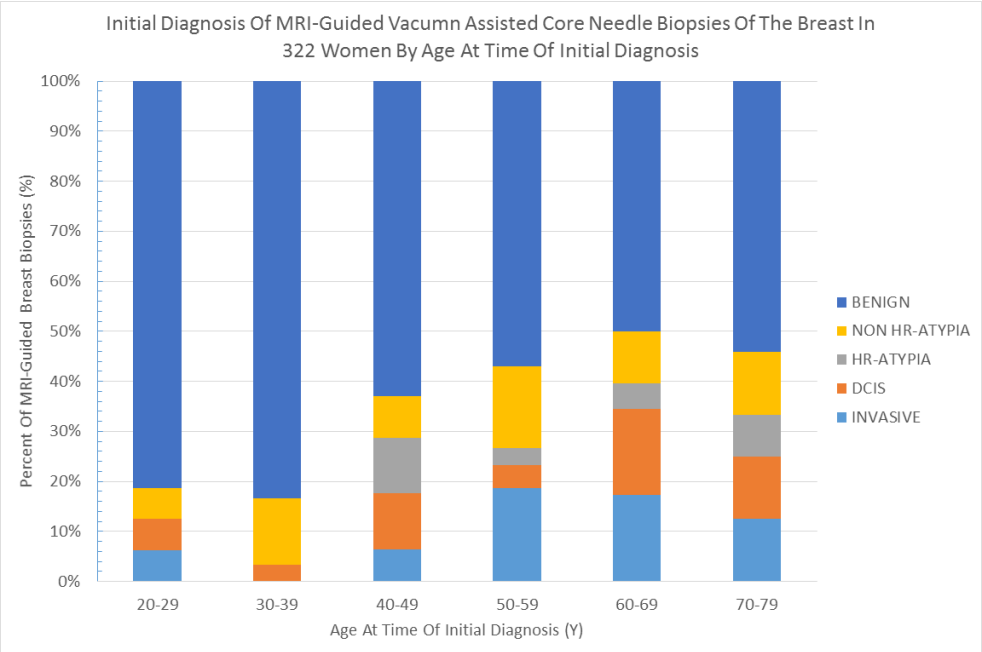
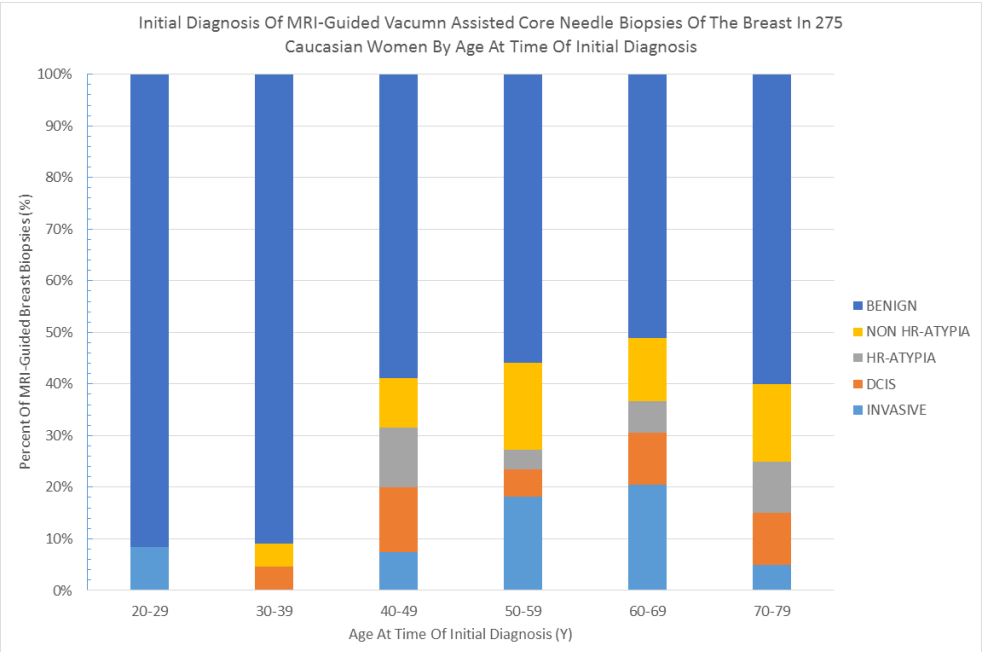


Figure 2 - 250



Conclusions: Based off of the current cohort, the overall diagnostic yield of malignancy and high-risk epithelial atypia in MRI guided biopsies of non-palpable breast lesions is marginal. While the potential exists to detect lesions much earlier than conventional techniques, benign findings are routinely biopsied.

251 An Appraisal of Clinicopathologic Parameters Associated with Response to Neoadjuvant Treatment in HER2 Positive Breast Cancer

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Disclosures: Juan Rong: None; Farnaz Hasteh: None; Oluwole Fadare: None; Somaye Zare: None

Background: Neoadjuvant HER2 targeted therapies have shown significant effectiveness in patients with HER2 amplified breast cancers; however, response to treatment may vary among patients. The aim of this study is to evaluate any possible correlation that may exist between routinely evaluable clinicopathologic parameters and response to neoadjuvant HER2 targeted therapy.

Design: Clinicopathologic data were reviewed on 118 patients with HER2 positive breast cancers that underwent neoadjuvant HER2 targeted therapy and surgical excision from 2014 to 2018 at a single institution. Treatment responses were evaluated on the excisional specimens using the MD Anderson residual cancer burden (RCB) classification. Cancers with pathologic complete response (PCR) or residual cancer burden-I (RCB-I) were classified as “responder”, and others as “non-responders”. The two groups were compared regarding a variety of clinicopathologic parameters, including patient age, tumor Nottingham grade (1/2+ vs 3+), HER2 IHC status (3+ vs others), HER2 copy numbers, HER2/CEP17 ratio, ER and PR status.

Results: Of the 118 cases, 41 (34.75%) were responders and 77 (65.25%) were non-responders. Responders and non-responders were similar in age (mean 53.5 years vs 51 years, p=.28). 92.6% of the responders showed HER2 3+ expression by IHC, compared with 54.5% of the non-responders (p<.001). In the responders group, 48.8% were ER positive and 29.2% were PR positive (p=0.003); in the non-responders group, 75.3% were ER positive and 61% were PR positive (p=0.001). The responders and non-responders showed comparable rates of grade 3 tumors. 97.5% of the responders had a HER2 copy number of >6, versus 65% of the non-responders (p<0.001). Only one patient had a HER2/CEP17 ratio of <2 in the responders group versus 12 in the non-responders group (p=0.029). The median HER2 signals/cell was >20 copies and the median HER2/CEP17 FISH ratio was 7.2 in the responders group. Corresponding values for the non-responders group were 7.1 HER2 signals/cell and a HER2/CEP17 FISH ratio of 3.

Conclusions: 3+ HER2 protein overexpression, high HER2 copy number, and ER/PR negativity are the primary predictors of response to neoadjuvant HER2 targeted treatment. Clinicopathologic features may help to predict response to neoadjuvant therapy in HER2 positive patients.

252 Neo-Adjuvant Anti-HER2 Therapy in Breast Cancer: Predictors of Pathological Complete Response and Survival

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Disclosures: Tricia Rood: None; Prasad Koduru: None; Sunati Sahoo: None; Helena Hwang: None; Yan Peng: None; Yisheng Fang: None; Venetia Sarode: None

Background: Pathologic complete response (pCR) is a marker for improved survival in HER2 positive breast cancer patients. Factors associated with pCR have not been well defined, especially in relation to level of HER2 overexpression and clinical outcome.

The purpose of this study was to identify factors that predict pCR among patients with HER2 positive breast cancer and to determine if there was an association between level of HER2 overexpression and survival.

Design: We retrospectively reviewed HER2+ breast cancer patients treated with neoadjuvant anti-HER2 and standard chemotherapy. Biomarkers (ER, PR, HER2, Ki67) were performed on all core biopsies using immunohistochemistry. HER2 FISH was performed on all IHC 2+ and most IHC 3+ cases. Scoring of biomarkers was performed according to ASCO/CAP guidelines. Pathologic response was evaluated after definitive surgery using standard gross and microscopic evaluation. pCR was defined as ypT0/is pN0. Follow-up information was obtained from electronic chart review. Survival was determined by log rank test.

Results: We identified 257 HER2 positive breast cancer patients; 99 (38.5%) achieved pCR and 158 (61.4%) had partial response. In univariate analyses, pCR was strongly associated with HER2 IHC 3+ (87%) vs HER2 IHC 0, 1+, and 2+ that were amplified by FISH (12%), (p<0.001), higher HER2 gene copy number (p=0.001) and HER2/CEP17 ratio (p=0.004), ER negative status (p<0.001), lower ER expression (p=0.002), PR negative status (p<0.001) and higher KI67 (p=0.033). Patients treated with Herceptin and Perjeta achieved pCR in 44% vs 34.7% with Herceptin alone (p=0.250). In multivariate analyses, HER2 IHC 3+ (p<0.001) and negative hormone receptor (HR) (p <0.001) status were independently associated with pCR.

The median follow-up was 36.3 months (9.5-198 months). Although the DFS (p=0.054) and RFS (p=0.055) was better in the pCR group, no significant difference was observed in the OS between the pCR and non-pCR groups (p=0.37). When pCR group was stratified by low HER2/CEP17 ratio (<6) versus high (≥6), the former had significantly better OS (p=0.045), DFS (p=0.0037) and RFS (p=0.0037).

Comparisons of the clinical and pathological characteristics between pCR and non-pCR groups (n = 257)			
Variables	**pCR (n = 99)**	**non-pCR (n = 158)**	**P value**
Age, years			
< 40	15 (15.2%)	26 (16.5%)	0.918
≥ 40	84 (84.8%)	132 (83.5%)	
BMI			
< 29	49 (50.0%)	84 (53.5%)	0.678
≥ 29	49 (50.0%)	73 (46.5%)	
Menopausal status			
Pre	40 (42.6%)	72 (46.8%)	0.608
Post	54 (57.4%)	82 (53.2%)	
Tumor size, cm			
< 2	18 (21.2%)	30 (23.1%)	0.873
≥ 2	67 (78.8%)	100 (76.9%)	
Mean ± SD	3.8 ± 2.5	3.9 ± 3.0	0.999
Tumor grade			
1	2 (2.1%)	3 (2.0%)	0.465
2	26 (26.8%)	52 (34.0%)	
3	69 (71.1%)	98 (64.0%)	
Nodal status			
Negative	14 (21.9%)	17 (15.9%)	0.436
Positive	50 (78.1%)	90 (84.1%)	
HER2 (IHC)			
0 and 1+	2 (2.0%)	9 (5.8%)	< 0.001
2+	11 (11.1%)	49 (31.4%)	
3+	86 (86.9%)	98 (62.8%)	
HER2 copy number			
Mean ± SD	18.4 ± 8.5	13.9 ± 7.6	0.001
HER2 ratio			
< 6	27 (41.5%)	63 (52.1%)	0.224
≥ 6	38 (58.5%)	58 (47.9%)	
Mean ± SD	7.4 ± 3.5	5.8 ± 3.3	0.004
ER			
Negative	57 (57.6%)	56 (35.7%)	< 0.001
Positive	42 (42.4%)	101 (63.3%)	
Percent positive	55.7 ± 39.1	77.9 ± 28.4	0.002
PR			
Negative	73 (73.7%)	74 (47.1%)	< 0.001
Positive	26 (26.3%)	83 (52.9%)	
Percent positive	34.4 ± 32.6	44.8 ± 35.1	0.180
Ki67 index	52.8 ± 21.7	47.3 ± 23.2	0.033
Anti-HER2 therapy	25 (26.3%)	47 (34.3%)	0.250
Herceptin	70 (73.7%)	90 (65.7%)	
Herceptin and Perjeta			

Conclusions: Although high HER2 expression was a predictor of pCR, the long term outcome was significantly better for patients with lower HER2 ratio who achieved pCR compared to those with higher ratio. This suggests that high HER2 expression could be a poor prognostic factor even though it was associated with initial high sensitivity to anti-HER2 therapy.

253 Insight into Utility and Impact of Immunohistochemistry in Evaluating Microinvasion in Breast Core Needle Biopsies

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Disclosures: Sudarshana Roychoudhury: None; Ugur Ozerdem: None; Dana Warfield: None; Thaira Oweity: None; Pascale Levine: None; Osvaldo Hernandez: None; Farbod Darvishian: None

Background: Diagnosis of microinvasion (MI) in breast core needle biopsy (CNB) can be challenging particularly in a background of carcinoma in situ (CIS) involving sclerosing lesion with periductal fibrosis and lymphocytic infiltrate. Immunohistochemical stains (IHC) for myoepithelial cells aid in confirming MI. Surgical management of MI deviates from CIS as the former includes sentinel lymph node biopsy (SLNB) while the latter typically includes SLNB only when total mastectomy (TM) is planned. We investigated the utility of IHC in diagnosing MI in our CNBs and its impact on final histopathology on surgical excision.

Design: We conducted a search for cases of CIS with foci suspicious for MI, in which IHC for calponin and p63 was used to confirm MI (defined as invasive carcinoma ≤1 mm) between January 2010 and June 2019. CIS included ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). MI cases diagnosed based on routine histology were also collected for the same time period. Only cases with follow up excision data were included. Cases with synchronous invasive carcinoma were excluded. Clinicopathologic data including age, size, laterality, resection type, SLNB status and biomarker profiles were compared. Graphpad Prism software was used for statistical analysis.

Results: We identified 106 cases of CIS (102 DCIS, 4 LCIS), where IHC was used to confirm MI (MI-IHC hereafter). Mean age was 58 years. Of the 106 cases MI-IHC was identified in 24 cases (23%). See table. All 24 MI-IHC cases had SLNB (100%). Of the 82 CIS cases, 39 had SLNB (48%). Relative risk of finding invasive carcinoma/MI on resection in MI-IHC was 1.8 (p=0.03) compared to CIS. There was no correlation between the biomarker profile with the resection outcome in either CIS (p=0.5, Fisher's exact test) or MI-IHC cases (p=3.4, Chi-square test). We identified 7 cases of MI, diagnosed on routine histology without IHC, of which 5 (71%) had invasive carcinoma/MI and 2 (29%) had CIS or no residual carcinoma on resection. Mean size of invasive carcinoma and CIS on resection in this group was 11 mm and 25 mm, respectively. The resection outcome between MI-IHC and MI based on routine histology was not significant (p=0.6).

CNB	Surgical Excision						
	Invasive carcinoma/MI	Mean size - invasive carcinoma/MI (mm)	CIS/No carcinoma	Mean size – CIS (mm)	TM	L	SLN+
CIS (n=82)	26 (32%)	2	56 (68%)	25	30 (37%)	52 (63%)	3 (8%)
MI-IHC (n=24)	14 (58%)	2.5	10 (42%)	17	12 (50%)	12 (50%)	0 (0%)
p value	0.03	0.13*	0.3	0.04*	0.2	0.2	0.3

CIS: Carcinoma in situ; CNB: Core needle biopsy; SLN: Sentinel lymph node; MI: Microinvasion; IHC: Immunohistochemistry; p value by Fisher's exact test; * p value by Mann-Whitney test; TM: Total Mastectomy; L: Lumpectomy

Conclusions: IHC helped diagnose MI in CNB for CIS in 23% of cases. Compared to CIS, the diagnosis of MI-IHC carried a relative risk of 1.8 in finding invasive carcinoma/MI on resection. There was no difference in the significance of the method used for the diagnosis of MI.

254 Utility of E-cadherin and p120 Stains in Evaluating Lobular Lesions: Should We Do One Stain or Both?

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Disclosures: Brandon Say: None; Megan Troxell: None; Robert West: None; Gregory Bean: None; Kimberly Allison: None

Background: While the distinction between ductal and lobular lesions can often be made on H&E, E-cadherin and p120 immunostains are frequently used as a diagnostic aid. This is most critical for in situ carcinomas, since the clinical management of DCIS and LCIS is very different. However, the most cost effective and timely staining algorithm has not been established. Should both of these stains be routinely used as a panel, or should one be ordered first (and if so which one)?

Design: 144 cases on 2 tissue microarrays (TMA) containing ductal and lobular lesions were stained with both p120 and E-cadherin. Cases were semiquantitatively scored for their cytoplasmic (C) and membranous (M) staining (negative (0), equivocal/uninterpretable (1), weak positive (2), strong positive(3)), special patterns (e.g., granular, crush, dot-like or incomplete membranous) and internal controls. On the basis of single stain evaluation, the pathologist was asked to make a diagnosis (ductal v lobular v indeterminate), score their confidence level, and report whether they would require the other stain for diagnosis.

Results: 92 cases of invasive carcinoma, 28 cases of CIS and 5 cases of normal breast were evaluated. In 65% of cases a diagnosis of ductal vs lobular could be made (confidence 2-3) for E-cadherin alone and 48% for p120. In 52% of cases it was indicated that the other stain should be reviewed for p120 and 36% for E-cadherin. For the 64 cases that were indeterminate on p120, E-cadherin would have been definitive 45% of the time. Conversely, for the 45 cases that were indeterminate on E-cadherin, the p120 stain would have been definitive 11% of the time. However, in 88% of cases of CIS only, a ductal vs lobular diagnosis could be made (with confidence 2-3) for p120 alone and 82% for E-cadherin alone. In 12% of cases it was indicated that the other stain should be reviewed for p120 and 19% for E-cadherin. Scores of C0M0 for E-cadherin and C3M0 were associated with high confidence using one stain alone. Scores of C0M1, C1M1, C2M1, C2M2 and C3M2 as well as variable and incomplete staining patterns were associated with low confidence.

Conclusions: Our data support starting with either p120 or E-cadherin for cases of CIS where morphologic features are ambiguous; both are not usually necessary. Invasive carcinomas with indeterminate features on TMA often remain indeterminate after dual staining. Data using whole stained sections will also be analyzed for confirmation of these initial TMA-based results.

255 Comparison of PAM50 and Oncotype-DX Risk Scores and Sub-Signatures for Prediction of Tumor Response Following Neoadjuvant Chemotherapy of Luminal-Subtype Breast Cancer

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Disclosures: Alexandra Schulz: None; Felix Kommoss: None; Esther Herpel: None; Elisa Braun: None; Carlo Fremd: None; Jörg Heil: None; Hans-Peter Sinn: None

Background: The PAM50 ROR score and the OncotypeDX recurrence score (RS) were shown to be of prognostic and predictive significance for adjuvant therapy in ER/PR-positive and HER2-negative breast cancer. Both tests contain sub-signatures with proliferation- and hormone receptor-related genes, but also apoptosis-related and other signatures. We studied the power of gene expression signatures in the pre-treatment core biopsy for prediction of pathologic complete response (pCR) defined as no invasive tumor residues in the specimens collected at surgery after neoadjuvant chemotherapy (ypTis/0 ypN0).

Design: A cohort of patients who had received a NACT for unilateral primary breast cancer at a single institution (time period: 2003 - 2012) were analyzed retrospectively. A total of 163 samples were selected which met the selection criteria of estrogen-receptor positive and HER2-negative disease, sufficiency of RNA in the core biopsy and passed RNA quality control criteria. RNA analysis was performed by measuring a custom panel of 269 breast cancer related genes and 11 housekeeping genes using the nCounter platform (Nanostring® Technologies, Seattle CA). Data normalization and statistical calculations were done using R Version 3.6.1.

Results: ROC cutpoint analysis revealed similar predictive scores for the ROR-score (cutpoint: 50.4, PPV: 0.23, RR=2.00) and the RS (cutpoint: 52.0, PPV: 0.25, RR=2.12). The proliferation signature of the ROR-score had a similar significance as the ROR-score itself (PPV: 0.25, RR=2.20). With the OncotypeDX, also its proliferation signature was predictive for pCR (PPV: 0.22, RR=1.88). Other sub-signatures of the ROR- or RS-scores were not significant for prediction of pCR. Differential gene expression analysis for the whole data set of 269 genes was significant for 36 upregulated genes in a logistic model for pCR vs. non-pCR (adjusted p-value < 0.05). Here, the most significantly predictive genes for pCR were two checkpoint kinases, CHEK1 (PPV: 0.33, RR=2.8) and PIM2 (PPV: 0.39, RR= 3.38), both involved in DNA damage response pathway and regulation of mitosis. Another 34 genes in the core biopsy were significantly repressed with regards to pCR, among them several genes of the estrogen-pathway such as ANKRD30A (PPV: 0.36, RR= 3.13) and TFF3 (PPV: 0.35, RR=3.02).

Figure 1 - 255

ROR-score (PAM50) and sub-signatures	RR	PPV	NPV	p-value
Risk of recurrence score (50 genes)	2.00	0.23	0.98	0.024
Proliferation (18 genes)	1.98	0.23	0.96	0.031
HER2 signaling (2 genes)	0.68	0.08	0.40	0.345
Cytoskeletal Function (4 genes)	0.60	0.07	0.88	0.744
Apoptosis (2 genes)	0.79	0.09	0.60	0.710
RTK and Prolif. Signaling (3 genes)	1.02	0.12	0.89	1.000
ECM & Adhesion (5 Genes)	0.76	0.09	0.87	0.813
Cell Division (3 Genes)	1.73	0.20	0.98	0.076
Hormone Signaling (3 Genes)	0.91	0.11	0.50	1.000
WNT Pathway (2 Genes)	0.70	0.08	0.84	0.526
Metabolism (4 Genes)	0.72	0.08	0.87	0.644
Ubiquitin Ligase (1 Gene)	1.13	0.13	0.93	0.701
Unclassified (3 Genes)	0.85	0.10	0.80	0.852

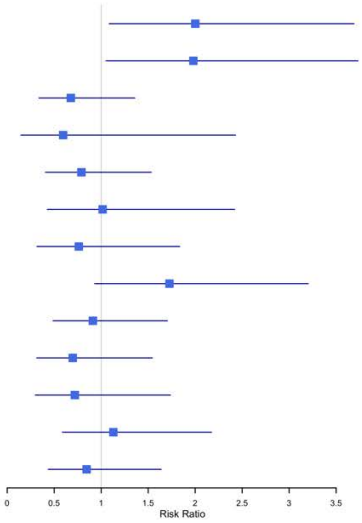
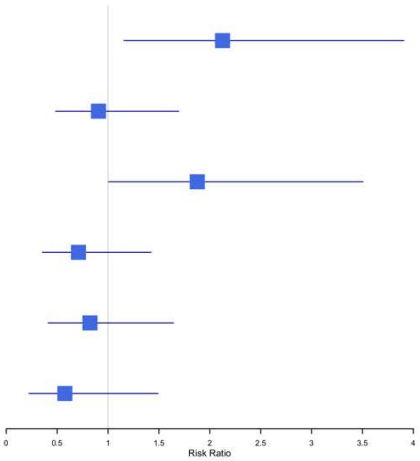


Figure 2 - 255

RS (OncotypeDX) and sub-signatures	RR	PPV	NPV	p-value
Recurrence score (16 genes)	2.12	0.25	0.98	0.013
Estrogen-related (4 genes)	0.91	0.10	0.00	1.000
Proliferation (5 genes)	1.88	0.22	0.97	0.040
HER2 signaling (2 genes)	0.71	0.08	0.65	0.446
Invasion (2 genes)	0.82	0.10	0.84	0.703
Others (3 genes)	0.58	0.07	0.85	0.351



Conclusions: Gene expression analysis may significantly improve prediction of neoadjuvant chemotherapy in pretreatment core biopsies, with proliferation and estrogen-pathway associated genes playing the major roles.

256 Pathways Associated with Invasion in Encapsulated Papillary Carcinoma of the Breast: Genomic and Transcriptomic Analysis

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Disclosures: Christopher Schwartz: None; Alireza Khodadadi-Jamayran: None; Adriana Heguy: None; Matija Snuderl: None; Paolo Cotzia: None; George Jour: None; Farbod Darvishian: None

Background: Encapsulated papillary carcinomas (EPC) of the breast is a variant of papillary carcinoma that are confined to a cystic space, surrounded by a fibrous capsule and lack the myoepithelial coat. Despite the latter finding, it is recommended that EPC be staged as pTis due to its indolent course. Concurrent frank invasive carcinomas are staged commensurate with their size. We sought to investigate the

molecular pathways differentially expressed in pure EPC and EPC with frank invasion at the genomic and transcriptomic level. In addition, we compared EPC with its corresponding invasive ductal carcinoma (IDC) at the transcriptomic level.

Design: We selected 3 cases of pure EPC (C1-C3) and 3 cases of EPC (C4e-C6e) with corresponding IDC (C4i-C6i).We performed whole transcriptome analysis on laser-capture microdissected samples from formalin-fixed, paraffin-embedded tissue. We used CloneTech Mammalian stranded pico kit for sequencing RNA. KEGG pathway analysis and Gene Ontology (GO) analysis was performed using the cluster Profiler R package (v3.0.0) and Database for Annotation, Visualization and Integrated Discovery. DNA analysis was performed using our in-house next generation sequencing hybrid capture covering 580 genes on C1-C3 and C4e-C6e.

Results: There were 5 female and 1 male patients. The mean age was 73 years (range 62-90). All cases were hormone receptor positive. C4e-C6e showed upregulation of *NTRK2* and *MAGI2* (lg2FC= 3.14 and lg2FC = 3.0 fold, respectively) and downregulation of *PRKACB* (lg2FC= -4.4) compared to C1-C3 on RNAseq. C4i-C6i showed upregulation of collagen-related genes (*COL10A1, COL11A1, COL14A1, COL16A1, COL1A1, COL3A1, COL8A1*) (lg2FC range: 6.28 fold change) and ADAM12/ADAMTS2 (lg2FC=6.2 and lg2FC= 6.9 fold change) compared to C4e-C6e (FDR =0.014). Pathway analysis showed upregulation of collagen fibril organization and extracellular matrix organization pathways in C4i-C6i compared to C4e-C6e and upregulation of kinase activity pathway (GO: 0016301) in C4e-C6e compared to C1-C3.Recurrent *PIK3CA* hotspot non-synonymous mutation was identified in C3, C4e, C5e and C6e (c.G1633A in C5 and c.A3140G in C3, C4 and C6).

Conclusions: Our findings suggest that kinase and matrix metalloproteinase pathways contribute to EPC with invasion compared to pure EPC cases. Furthermore, enrichment of collagen-related genes in IDCs compared to their corresponding EPC suggest a synergistic potential with the aforementioned pathways. Mechanistic studies are warranted to validate the findings.

257 Adenoid Cystic Carcinoma of the Breast: A Single Institution Experience with Emphasis on Solid Variant with Basaloid Features

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Disclosures: Christopher Schwartz: None; Edi Brogi: None; Fresia Pareja: None; Sujata Patil: None; Britta Weigelt: None; Jorge Reis-Filho: None; Hannah Wen: None

Background: Adenoid cystic carcinoma of the breast (AdCC) is a rare histologic subtype of breast cancer (BC) that is usually of triple-negative (TN) phenotype, but has favorable prognosis, unlike most TNBC. A solid variant of AdCC with basaloid features (AdCC-SB) has been described and features a higher rate of lymph node metastases than conventional AdCC (AdCC-C). The long-term outcome of AdCC-SB patients has not been fully characterized.

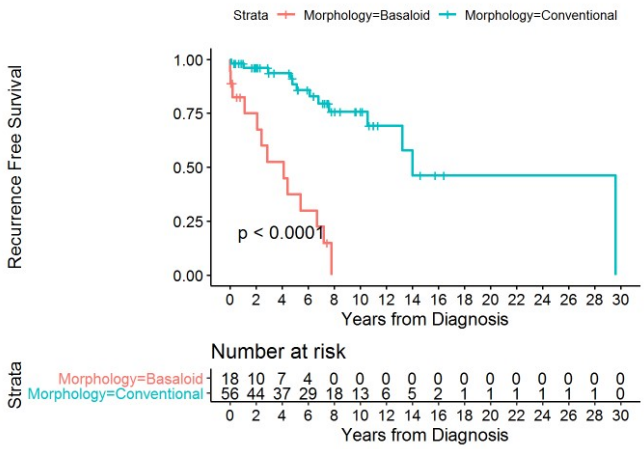
Design: A search of our pathology database identified patients with AdCC of the breast diagnosed between 1988-2019 at our Center. We compared clinicopathologic features and outcomes of AdCC-C and AdCC-SB, classified based on pathology reports. Statistical analysis was performed using Fisher's exact test for categorical variables and t-test for continuous variables. Recurrence free survival (RFS) was analyzed using log rank test.

Results: A total of 104 AdCCs (79 AdCC-C; 25 AdCC-SB) were identified (Table 1). 69 cases were triple negative (87%), 10 cases had low ER expression (13%). Patients with AdCC-SB were significantly older than AdCC-C (mean age at diagnosis 64 years vs 58 years, respectively; p=0.048), and had larger tumors (size 2.3 cm and 2.0 cm, respectively; p=0.290). AdCC-SB had higher tumor grade (grade 3; 66% vs 17%, p=.007), and more frequent lymphovascular invasion (20% vs 3.8%, p=0.02). The reported rate of axillary lymph node metastases was higher in AdCC-SB, but not significant (8% vs 2.5%, p=0.243). There were no significant differences in treatment between AdCC-SB and C-AdCC groups in terms of surgery (breast conserving surgery (BCS, 81% vs. 73%) vs. total mastectomy (TM, 19% vs. 27%, p=0.573) , radiation treatment (RT, 79% vs. 65%, p=.393), chemotherapy (28% vs 42%, p=.269) or endocrine treatment (1.8% vs 0, p=1). Follow-up data were available for 57 AdCC-C (median follow up, 61.5 months) and 19 AdCC-SB (median follow up, 42 months). Patients with AdCC-SB had a markedly reduced RFS compared to AdCC-C (median RFS, 4.1 months vs. 14 months, p<0.0001, Figure 1).

	Conventional (n=79)	Basaloid (n=25)	p value
Mean age at diagnosis (years)	58	64.25	0.048
Mean tumor Size (cm)	1.98	2.25	0.290
Tumor Grade*			0.007
I-II	20/24 (29%)	4/12 (8.3%)	
III	4/24 (16.6%)	8/12 (66%)	
LVI	3/79 (3.8%)	5/25 (20%)	0.019
PNI	7/79 (8.9%)	5/25 (20%)	0.152
Nodal metastasis at diagnosis	2/79 (2.5%)	2/25 (8%)	0.243
Triple negative	50/59 (85%)	19/20 (95%)	0.436
ER-positive	9/59 (15%)	1/20 (5%)	0.436
Surgery**			0.573
BCS	51/70 (73%)	17/21 (81%)	
TM	19/70 (27%)	4/21 (19%)	
RT ***	37/57 (65%)	15/19 (79%)	0.393
Endocrine therapy ***	1/57 (1.8%)	0/19 (0%)	1.000
Chemotherapy ***	16/57 (28%)	8/19 (42%)	0.269

*Tumor grade was available for 24 conventional and 12 basaloid AdCC; ** Type of surgery data was available for 70 conventional and 21 basaloid AdCC; *** Adjuvant treatment information was available for 57 conventional and 19 basaloid AdCC

Figure 1 - 257



Conclusions: Compared to conventional AdCC, AdCC-SB has higher Nottingham grade and more frequent lymphovascular invasion, a higher rate of lymph node metastases at diagnosis and distant metastasis at follow-up, with significantly worse outcome. Histologic subtyping of AdCCs should be considered for treatment decision-making. A molecular analysis of these cases is ongoing.

258 Concordance of Breast Cancer Biomarker Status between Routine Immunohistochemistry/In Situ Hybridization and Oncotype DX qRT-PCR with Investigation of Discordance, a Study of 591 Cases

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Disclosures: Haley Sechrist: None; Akisha Glasgow: None; Philip Bomeisl: *Consultant*, PathAI; Hannah Gilmore: None; Aparna Harbhajanka: None

Background: Patients with estrogen receptor (ER) positive/HER2 negative breast cancer with high recurrence risk can benefit from adjuvant chemotherapy. Oncotype DX (ODX) assists in predicting high risk cancers, reporting a recurrence risk score (RS) and scores for ER, progesterone receptor (PR), and HER2. This study examines the discordance of biomarker status between ODX and routine immunohistochemistry (IHC)/in situ hybridization (ISH) and evaluates clinicopathologic features of discordant cases.

Design: A total of 591 cases were reviewed to compare ER, PR, and HER2 status between IHC/ISH and ODX. RS and clinicopathologic features including chemotherapy, recurrence, and metastasis were compared between concordant and discordant cases. H&E slides from ER discordant cases were reexamined.

Results: Concordance rate was high between ODX and IHC for ER status (580/591, 98.1% concordant) and moderate for PR status (512/591, 86.6% concordant). There were 11 ER discordant cases which were all ER+ by IHC but ER- by ODX. All ER discordant cases were high risk by ODX as ER status influences overall RS. Histologically, all of these cases were grade III invasive ductal carcinoma (IDC), except one case diagnosed as grade I IDC with apocrine features. Although this case was grade I and ER/PR positive by IHC, this patient received chemotherapy due to the high RS. Of 79 PR discordant cases, 60 were PR+ by IHC but PR- by ODX. 584 cases had HER2 results, with high negative agreement (580/582, 99.7% concordant). Two cases were HER2- by IHC/ISH but equivocal by ODX. Two cases were HER2+ by ISH but HER2- by ODX, one case testing HER2- by repeat ISH on excision. Mean RS for ER discordant cases was higher than for ER concordant cases (48.0 versus 17.1, p value<0.0001). Similarly, mean RS for PR discordant cases (IHC+/ODX-) was higher than for PR concordant cases (27.2 versus 16.7, p value<0.0001). However, there was no significant difference in recurrence or metastasis between ER/PR concordant and discordant cases.

	Age (yr, mean ± SD)	Oncotype Score (mean ± SD)	Recurrence	Metastasis	Chemotherapy
ER Concordant N=580	60.4±10.2	17.1±9.1	21 (3.7%)	15 (2.7%)	118 (20.7%)
ER Discordant N=11	56.4±7.5	48.0±11.8	1 (10.0%)	0 (0.0%)	9 (90.0%)
ER Comparison p value	0.194	<0.0001	0.321	1.0	<0.0001
PR Concordant N=512	59.9±10.5	16.7±9.5	17 (3.4%)	15 (3.0%)	98 (19.5%)
PR Discordant (IHC+/ODX-) N=60	62.8±6.2	27.2±10.7	5 (8.6%)	0 (0.0%)	26 (44.1%)
PR Discordant (IHC-/ODX+) N=19	62.8±10.7	16.1±4.2	0 (0.0%)	0 (0.0%)	3 (15.8%)
PR Comparison p value	0.075	<0.0001 (<0.0001*)	0.094	0.305	<0.0001

Clinicopathologic Comparisons of ER/PR Concordant and Discordant Cases. Age, Oncotype risk score, recurrence, metastasis, and treatment with chemotherapy were compared between ER concordant and discordant cases (ER Comparison p value) and between PR concordant and discordant cases (PR Comparison p value). PR discordant cases positive by IHC and negative by ODX are denoted IHC+/ODX-, while cases negative by IHC and positive by ODX are denoted IHC-/ODX+. Statistical analysis was done using T-test or one-way ANOVA for numerical data (Oncotype score and age) and chi square analysis for categorical data (chemotherapy, recurrence, and

metastasis). P value <0.0001* indicates a statistically significant difference in mean Oncotype score between PR concordant and IHC+/ODX- PR discordant cases by Bonferroni post hoc analysis.

Conclusions: ODX and IHC displayed high ER concordance and moderate PR concordance, with more sensitive detection by IHC. HER2 concordance between ODX and IHC/ISH was high for HER2- cases, while the HER2+ case by ISH was reported as HER2- by ODX, suggesting more sensitive detection by ISH. High RS of discordant cases suggest possible risk overestimation. Therefore, therapeutic decisions for discordant cases should be based on clinical and pathological correlation and not Oncotype score alone.

259 Intraoperative Evaluation of the Nipple/Subareolar Tissue During Nipple Sparing Mastectomy: Accuracy, Pathological Correlation and Clinical Significance

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Disclosures: Antonio Serrano: None; Farbod Darvishian: None; Ugur Ozerdem: None; Diana Nimeh: None; Paolo Cotzia: None; Stella Gordin: None

Background: Intraoperative evaluation of the nipple/subareolar tissue (N/SAT) has been used by surgeons to assess for occult nipple involvement by malignancy and guide the decision-making process for nipple preservation during nipple sparing mastectomies (NSM). The aim of our study is to evaluate significance and accuracy of frozen section (FS) results compared to final pathology/permanent sections.

Design: We retrospectively reviewed records of patients that underwent NSM with FS of the N/SAT from 2014 to 2018. Positive FS or final pathology results include atypical hyperplasia, in situ and invasive carcinoma.

Results: Over a 5-year period a total of 339 NSM cases utilized FS to evaluate the N/SAT. Of the total 339 cases, 85(25%) were prophylactic and 254(75%) were therapeutic mastectomies. All 85 prophylactic mastectomies were negative (benign) on FS and final diagnosis. Among 254 therapeutic mastectomies, 217(85.4%) showed negative (benign) intraoperative FS with concordant benign final pathology; 22(8.7%) showed positive intraoperative FS with concordant positive final pathology; 15(5.9%) were false negative (benign) on FS and positive on final permanent sections (figure 1). Positive results consisted of atypical ductal or lobular hyperplasia (5, 27.8%), in situ ductal or lobular carcinoma (11, 61.1%) and invasive carcinoma (1, 5.6%). One false positive case showed “atypical intraductal proliferation” at FS and was diagnosed as intraductal papilloma on final pathology; the nipple was not removed at the time of surgery. Of the 37 cases with positive final nipple pathology, 14(37.8%) had intraoperative resection of the nipple/areola complex (NAC), 9(24.3%) required an additional surgery for removal of NAC and 13(35.1%) had no additional procedures performed. Residual pathology was identified in 9(39.1%) of the resected NAC. In our patient cohort frozen section diagnosis has a sensitivity of 58.3%, specificity of 99.5%, positive predictive value (PPV) of 95.5% and negative predictive value (NPV) of 93.5%.

Figure 1 - 259

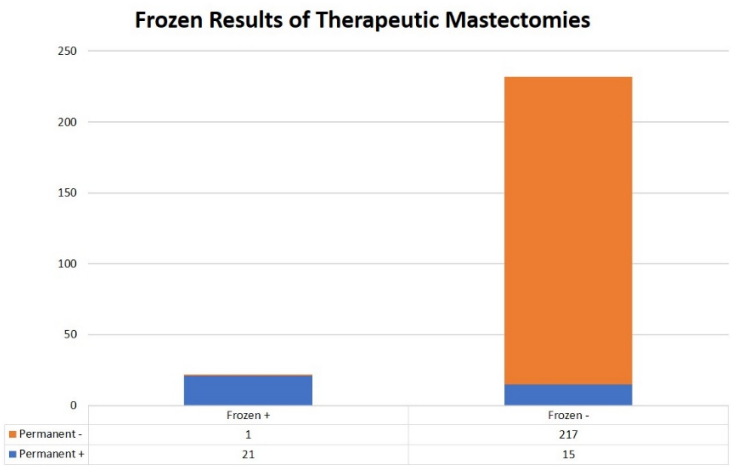

Figure 1. The breakdown of results for intraoperative frozen section diagnosis for all therapeutic mastectomy cases.

Conclusions: Intraoperative evaluation of the N/SAT is highly accurate (93.7%) and specific (99.5%) test that prevented additional surgical intervention in 8.7% of therapeutic mastectomies. At the same time, surgeons should be aware of the low/moderate sensitivity of intraoperative FS, which may be explained by processing artifacts, sampling or cautious approach not to overcall the FS findings.

260 Chondroid Matrix-Producing Metaplastic Carcinomas of the Breast are Genetically Distinct from Mixed Metaplastic Carcinomas with Chondroid Differentiation

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Disclosures: Eliah Shamir: None; Yunn-Yi Chen: None; Gregory Bean: None; Poonam Vohra: None; Melinda Sanders: None; Gregor Krings: None

Background: Metaplastic carcinomas of the breast (MC) are rare tumors with differentiation of neoplastic epithelium into squamous or mesenchymal-like elements. MC are usually triple negative (TNBC) but are morphologically and molecularly heterogeneous, and histotype may correlate with outcome. Whereas squamous (SqCC) and spindle cell carcinomas (SpCC) have frequent PI-3 kinase(K) and *TERT* promoter mutations, chondroid matrix-producing MC (CMPC) lack these aberrations. Mixed MC with multiple lineages often have chondroid differentiation (MMCD), but whether CMPC are biologically distinct from other MC with chondroid differentiation is unknown. We profiled CMPC and MMCD using capture-based next generation sequencing (NGS) and compared subtypes.

Design: DNA was extracted from 9 MMCD, 10 CMPC and matched normal tissue. MMCD included tumors with at least 3 lineages in 7/9 cases: chondroid (100%), spindle (100%), osseous (44%), squamous (44%) and rhabdomyoid (11%). CMPC were defined by accepted histologic criteria. NGS was performed targeting exons of 479 cancer genes, 40 introns and *TERT* promoter. Duplicate reads were removed computationally for allele frequency determination and copy number alteration (CNA) calling. Single nucleotide variants, insertions/deletions and CNA were evaluated. Genetics and clinicopathologic features were compared between groups and genetics were compared with TCGA TNBC (n=81).

Results: PI-3K pathway aberrations were identified in 89% MMCD, including *PIK3CA* (3/9), *PIK3R1* (3/9), *PTEN* (3/9), and *AKT1* (1/9), but were absent in CMPC (0%, p<.001). The single MMCD lacking PI-3K aberrations had a driver germline *BRCA1* mutation. *TERT* promoter mutations were present only in MMCD (33%) not CMPC (0%, p=.08). There was no difference in *TP53* mutation status (89% MMCD, 90% CMPC). Both subtypes were genomically unstable. Frequency of *PIK3CA*/*PIK3R1* (56% vs 20%), *PTEN* (33% vs 7%) and *RB1* (44% vs 12%) aberrations were higher in MMCD than TCGA TNBC (p=.01, p=.04, and p=.04, respectively) and similar to other non-CPMC MC subtypes. MMCD mean tumor size (8.6±6.3cm) was larger than CMPC (2.7±1.4cm, p=.01); other clinicopathologic features and outcomes were statistically similar.

Conclusions: CMPC are genetically distinct from MMCD. Along with published data, the results support classification of CMPC as a distinct MC subtype, and these tumors should be distinguished from other MC with chondroid differentiation. MMCD with spindle elements share PI-3K and/or *TERT* promoter aberrations with SpCC and SqCC.

261 Genetic and Immunohistochemical Characterization of Serous-Like Carcinomas of the Breast

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Disclosures: Eliah Shamir: None; Charles Zaloudek: None; Miriam Post: None; Yunn-Yi Chen: None; Gregor Krings: None

Background: Rare invasive breast carcinomas have serous-like morphologic features mimicking gynecologic serous carcinomas (SLBC). A recent study suggested SLBC are triple negative (TN) and clinically aggressive (Mod Pathol 2019;32:52-54). However, histopathologic and genetic characterization of SLBC is lacking, and it is unknown if these tumors represent a distinct subtype. We used capture-based next generation sequencing (NGS) and immunohistochemistry (IHC) to determine if SLBC have characteristic features.

Design: Nine SLBC were analyzed. DNA was extracted from 7 SLBC and matched normal tissue. NGS was performed targeting exons of 479 cancer genes, 40 introns, and TERT promoter. Duplicate reads were removed computationally for allele frequency determination and copy number alteration (CNA) calling. Single nucleotide variants, insertions/deletions and CNA were evaluated. IHC was performed on 8 SLBC for ER, PR, HER2, GATA3, mammaglobin, GCDFP-15, SOX10, PAX8, WT1, and p53.

Results: Mean age was 48 (range 27-67). Mean tumor size was 4.6±3.4 cm and 56% had ductal carcinoma in situ. No patients had gynecologic tumors. SLBC were histologically distinct, with anastomosing branched glands ± tufts or micropapillae in desmoplastic stroma. Eight had high nuclear grade (HNG) and were modified SBR grade 2 (4/8) or 3 (4/8); of these, 7 were TN and 1 was ER-/HER2+. All HNG SLBC had *TP53* mutations. Other aberrations included amplification of *CCNE1* (2/7), *CCND3/VEGFA*, *NOTCH2*, and *ERBB2* (1 each) and *CDK12* rearrangement (1/7). HNG SLBC had numerous CNA, including recurrent gains of 1q and distal 17q and loss of 5q, 8p and 14q (3 cases each). One SLBC was SBR grade 1/low nuclear grade and weakly ER+/HER2-. This tumor lacked *TP53* mutation and had *KRAS* hotspot mutation with inactivating mutations in *KDM6A*, *CREBBP*, *ARID1A* and *DNMT3A*. CNA analysis showed only 1q gain. All SLBC were GATA3+ and negative for GCDFP15, PAX8, and WT1. SOX10 was positive in 7/8 and negative in the HER2+ tumor. Mammaglobin was negative (6/8) or focally positive (2/8). P53 IHC correlated with *TP53* mutation status. Of 8 patients with follow-up (mean 48 months), 4 had nodal metastasis, 2 had local recurrence, 1 had distant metastasis and 2 died of disease.

		#1	#2	#3	#4	#5	#6	#7	#8	#9
Nuclear grade		3	3	3	3	3	3	3	3	1
mSBR grade		2	3	3	2	2	3	3	2	1
DCIS		NO	YES	NO	NO	YES	YES	NO	YES	YES
IHC	**ER**	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Weak Pos
	PR	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	HER2	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
	GATA3	Pos	Pos	Pos	Pos	Pos	Weak Pos	Pos	NA	Pos
	MGB	Neg	Focal	Focal	Neg	Neg	Neg	Neg	NA	Neg
	GCDFP-15	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NA	Neg
	SOX10	Pos	Pos	Pos	Neg	Pos	Pos	Pos	NA	Pos
	PAX8	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NA	Neg
	WT1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	NA	Neg
	p53	Aberrant (diffuse strong)	Aberrant (diffuse strong)	Neg	NA	Aberrant (diffuse strong)	Aberrant (diffuse strong)	Aberrant (cytoplasmic)	NA	wild-type pattern
NGS	***TP53* mutation**	YES	YES	YES	YES	YES	YES	NA	NA	NO
	Other pathogenic alterations	*CCNE1* amp	*CCNE1* amp	*CCND3/VEGFA* amp	*ERBB2* amp, *CDK12* rearrangement	*NOTCH2* amp, subclonal *PIK3CA* mut	None	NA	NA	*KRAS*, *KDM6A*, *CREBBP*, *ARID1A*, *DNMT3A* mut

Conclusions: SLBC comprise a unique histotype of breast cancers that are morphologically and genetically indistinguishable from gynecologic serous carcinomas. IHC, especially GATA3, PAX8, and WT1, is useful in the differential diagnosis. TN SLBC are genetically similar to other TN breast cancers.

262 HER2 Intratumoral Heterogeneity is Associated with Distal Metastasis in HER2-positive Breast Carcinoma

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Disclosures: Tiansheng Shen: None; Hiro Nitta: *Employee*, Roche Tissue Diagnostics; Anil Parwani: None; Zaibo Li: None

Background: Human epidermal growth factor receptor 2 (HER2) intratumoral heterogeneity (ITH) occurs with variable frequencies in breast cancers and has been reported as an independent predictive factor for the response to anti-HER2 neoadjuvant therapy. However, there have been few studies of its association with distal metastasis.

Design: Assessment of HER2 ITH was performed on whole tissue sections of pretreatment primary breast tumors from a cohort of 158 HER2-positive invasive breast carcinomas treated with anti-HER2 chemotherapy (neoadjuvant or adjuvant) and surgery. Both HER2 gene signal and protein expression were simultaneously evaluated by means of a single-slide dual assay, designated as a HER2 gene-protein

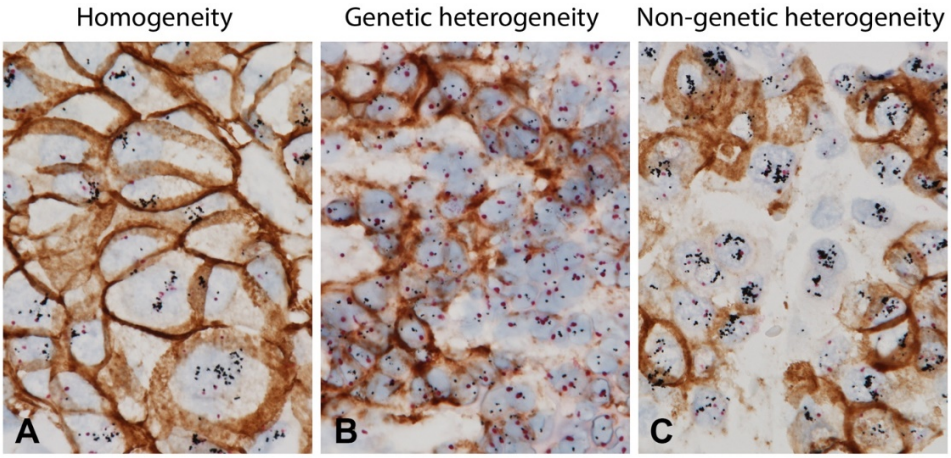
assay (GPA). HER2 ITH was categorized into genetic (mixture of HER2-positive tumor cells with both HER2 gene amplification and HER2 protein overexpression and HER2-negative tumor cells with negativity at both levels) and non-genetic ITH (only protein heterogeneity caused by discordant gene amplification and protein overexpression). Figure 1 shows representative images from cases with HER2 homogeneity, genetic heterogeneity, and non-genetic heterogeneity.

Results: The cohort was composed of 158 HER2-positive invasive breast carcinomas including 44 with and 114 without distal metastasis. Statistical analysis revealed distal metastasis was associated with higher Nottingham grade, larger tumor size, positive lymph node and negative estrogen receptor/progesterone receptor (ER/PR), but not age, neoadjuvant therapy, nuclear grade, HER2 immunohistochemistry (IHC), HER2 copy number or ratio. Fifty-seven cases (36%) showed HER2 ITH including 19 with genetic, 8 with both genetic and non-genetic, and 30 with non-genetic ITH. The presence of ITH or genetic ITH was associated with increased frequency of distal metastasis, but not non-genetic ITH (Table 1).

Table 1. Clinical and pathological results in HER2-positive cases with and without distant metastasis

		Total		Non-metastatic		Metastatic		p value
		#/median(average)	%/range	#/median(average)	%/range	#/median(average)	%/range	
Total cases		158		114		44		
Age (year) (median/range)		57	30-90	58	30-90	56	31-70	0.0566
Neoadjuvant therapy	Available	68		56		12		
	Complete response	41	60%	34	61%	7	58%	NS
	Residual tumor	27	40%	22	39%	5	42%	
Tumor size (cm) (median/range)		2.30	0.03-8.70	1.95	0.03-6.80	3.25	0.38-8.70	<0.0001
Lymph node	Available	147		110		37		
	Positive	41	28%	21	19%	20	54%	<0.0001
Grading	Nottingham grade	2.56	1-3	2.48	1-3	2.77	2-3	0.0038
	Nuclear grade	2.74	1-3	2.71	1-3	2.82	2-3	0.2444
ER/PR	ER+	89	56%	71	62%	18	41%	0.0150
	ER%	45.3%	0-100%	51%	0-100%	31%	0-100%	0.0156
	PR+	62	39%	51	45%	11	25%	0.0227
	PR%	19.3%	0-100%	22%	0-100%	12%	0-95%	0.0703
HER2 IHC	Available	155		113		42		NS
	HER2 IHC3+	127	82%	98	87%	29	69%	0.0749
HER2 FISH	Copy number	16.72	2.51-40.31	17.45	2.51-40.31	16	4.00-35.39	NS
	Ratio	6.04	0.93-22.98	6.05	1.13-22.98	5.05	0.93-15	NS
HER2 intratumoral heterogeneity (ITH)	Genetic ITH	19	12%	8	7%	11	25%	0.0040
	Genetic + non-genetic ITH	8	5%	5	4%	3	7%	NS
	Total genetic ITH	27	17%	13	12%	14	32%	0.0065
	Non-genetic ITH only	30	19%	22	19%	8	18%	NS
	All ITH	57	36%	35	31%	22	50%	0.0260

Figure 1 - 262



Conclusions: HER2 ITH analyses conducted with GPA method revealed that HER2 ITH is associated with distal metastasis in HER2-positive breast cancer patients.

263 Expression of FOXA1 and EZH2 in Breast Cancer and Association with Clinicopathological Features and TP53/RB1 Alterations

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Disclosures: Mingxia Shi: None; Shu Yang: None; Siyuan Cheng: None; Liurka Lopez: None; Xiuping Yu: None

Background: Forkhead box A1 (FOXA1), is receiving considerable attention because it controls downstream transcription of estrogen receptor (ER)-regulated genes. The enhancer of zeste homolog 2 (EZH2) is a transcriptional repressor involved in cell cycle regulation and has been linked to aggressive breast cancer. The aim of this study was to elucidate the correlation of FOXA1 and EZH2 expression, and evaluate their association with clinicopathological parameters and molecular pathways in breast cancer.

Design: The expression of FOXA1 and EZH2 was analyzed immunohistochemically in 143 patients with invasive breast carcinoma, and correlated with various clinicopathologic features and molecular subtypes. The mRNA expression of FOXA1 and EZH2 as well as mutations of P53 and RB1 were extracted from cBioportal-Breast Cancer METABRIC dataset, which contains microarray data and targeted sequencing data from primary breast tumors of 1904 patients. A bioinformatics analysis was performed.

Results: FOXA1 protein expression was inversely correlated with EZH2 protein expression (P =0.031). FOXA1 expression was demonstrated in 59% of invasive breast cancers. It correlated positively with expression of ER, PR, AR, luminal A subtype (p<0.001), and inversely correlated with tumor grade, Ki67 index (p< 0.001), p53 expression (p=0.013) and triple negative subtype (p <0.001). EZH2 expression was seen in 54% of invasive breast cancers. High EZH2 expression was significantly associated with high tumor grade, high Ki67 index, positive p53 expression, ER negativity, PR negativity, AR negativity, HER-2 enriched and triple negative breast cancer (p<0.001). Our bioinformatics analysis confirmed the inverse correlation between FOXA1 and EZH2 expression (p<0.001) in triple negative breast cancer. Our analysis has revealed that reduced FOXA1 expression or increased EZH2 expression correlates with basal and claudin-low subtypes of breast cancer. A strong association between expression of EZH2 and the percentage of tumors with alterations in TP53, RB1, or both (p<0.001) was demonstrated. Decreased FOXA1 expression was also associated with increasing percentage of tumors that have alterations in TP53, RB1, or both (p<0.01).

Conclusions: Our results show that FOXA1 expression is associated with favorable tumor characteristics and prognostic factors, while EZH2 expression is strongly associated with features of aggressive breast cancer and TNBC. Expression of both biomarkers is associated with alterations of TP53/RB1 pathways.

264 Mucocele-Like Lesions of the Breast: Correlating Core Biopsy with Excisional Biopsy Findings

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Disclosures: Yukiko Shibahara: None; Vivianne Freitas: None; Anna Marie Mulligan: None

Background: Mucocele-like lesions (MLL) are uncommon in breast biopsies. Although upgrade rates to carcinoma are reportedly low, excision is often performed to permit definitive diagnosis and exclude malignancy. We reviewed the spectrum of findings associated with MLLs identified on core biopsy in a major tertiary referral cancer center over a 19 year period and correlated these with excision diagnoses.

Design: All patients who had a biopsy diagnosis of MLL from Jan 1, 2000 to March 31, 2019 were identified. MLLs were classified as (i) MLL without atypia, (ii) MLL with atypia, (iii) MLL with suspicious (i.e. detached epithelium within mucin raising the possibility of invasive carcinoma) but non-definitive findings, (iv) MLL with ductal carcinoma in situ (DCIS). Patient age, imaging findings, method/gauge of biopsy, clinical history and follow-up were recorded.

Results: Sixty biopsies from 59 patients were identified (age range: 39 to 76 (mean 54) years). Stereotactic (n=50) or ultrasound (n=10) guided biopsies were performed targeting calcifications (n=56) or a mass (n=4). Excision was not performed in 10 patients. Table 1 summarizes the core biopsy and corresponding excisional diagnoses. Of 24 MLL without atypia on biopsy, 11 showed atypia in the excision (4 with flat epithelial atypia (FEA), 5 with atypical ductal hyperplasia (ADH), 2 with lobular neoplasia (LN)) and one patient, with increasing calcifications, had low grade DCIS with microinvasion on excision (upgrade to malignancy rate 4%). Of 22 patients with MLL with atypia (FEA n=3, ADH n=18, LN n=1), 4 (18%) had either invasive (n=1) or in situ (n=3) carcinoma on excision. In 3 patient's cores, detached epithelium within mucin was identified raising the suspicion for invasion. All 3 were confirmed to have invasive carcinoma on excision. Of 6 patients with invasion on excision, the radiologic findings were: mass (n=2), asymmetric density (n=1), cluster of cysts (n=1), suspicious calcifications (n=1) and indeterminate calcifications (n=1).

Table 1

Biopsy Diagnosis	Excision Diagnosis				Total
	No atypia	Atypia	DCIS	Invasive	
No atypia	12	11		1	24
Atypia	3	15	3	1	22
Suspicious*				3	3
DCIS				1	1
Total	15	26	3	6	50

*Suspicious but non-definitive on biopsy

Conclusions: MLL are exceptionally rare, even in high volume breast centers; however, they are associated with atypical hyperplasia in a high proportion of cases. In the absence of atypia, the rate of upgrade to malignancy is low which may obviate the need for routine excision, provided radiologic correlation is achieved. MLL with atypia on core biopsy require excision in view of the significant rate of malignant upgrade. The presence of a mass lesion on imaging may predict for malignancy in this setting.

265 Immunohistochemical Expression Patterns of CHK2, PTEN, ATM and PALB2 in Breast Cancers with Corresponding Deleterious Germline Mutation

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Disclosures: Kamaljeet Singh: None; Jessica Laprise: None; Jennifer Scalia Wilbur: None; Mark Zingarelli: None; Robert Legare: None; M. Ruhul Quddus: None; C. James Sung: None

Background: Deleterious germline mutations in *CHEK2*, *ATM, PALB2* & *PTEN* genes confer an increased breast cancer (BC) risk. Clinical features and family history based algorithms are employed to select BC patients for germline mutation testing. Immunohistochemical (IHC) expression of protein products of mutated high-risk genes has not been investigated in BC. We hypothesized that mutations in these 4 genes may lead to an abnormal IHC expression pattern of corresponding protein products in the tumor cells.

Design: BC patients with deleterious germline mutations in *CHEK2*, *ATM, PALB2* & *PTEN* were identified. The H&E stained slides were reviewed to identify tumor-containing formalin fixed paraffin embedded tissue blocks. Immunohistochemistry was performed using Dako staining platform. Primary antibodies for PALB2 (ab202970), ATM (2C1[1A10]), CHK2 (EPR4325), and PTEN (138G6) proteins were used for BCs with respective deleterious mutations. IHC expression was assessed in tumor cells and adjacent benign breast tissue.

Results: Total 26 BCs with 10 *CHEK2*, 9 *ATM*, 6 *PALB2* & 1 *PTEN* deleterious germline mutations were identified. One *PTEN* mutated case with ADH was included in the study. IHC staining was performed on 8 *CHEK2*, 7 *ATM*, 6 *PALB2* & 2 *PTEN* cases. Abnormal CHK2 IHC staining was identified in 7/8 (88%) BCs. Three distinct CHK2 IHC patterns were noted: 1) Strong diffuse nuclear positivity (Fig 1A) in 5/8 cases, 2) Null-pattern with no staining (Fig 1B) in 2/8 cases, & 3) Normal breast–like staining (Fig 1C) in 1 case. Four of 5 (80%) strong CHK2 staining tumors had missense mutations. Null-pattern was present with in 1 case each with a missense & a frameshift mutations. Mild to moderate diffuse nuclear IHC staining, similar to normal breast CHK2 IHC staining pattern was present in 1 case with frameshift mutation. Loss of nuclear/cytoplasmic PTEN IHC expression was noted in 2 *PTEN* mutated cases. In carcinoma case, PTEN loss was noted in high grade DCIS (Fig. 2A) as well as in ADH foci (Fig. 2B). ATM and PALB2 IHC expression pattern was similar in tumor cells and benign breast epithelium. Both ATM and PALB2 showed diffuse mild to moderate nuclear and cytoplasmic staining.

Details of the *CHEK2* germline mutations and CHK2 immunostaining patterns in eight breast cancers

	Type of mutation	cDNA location	Amino acid affected	CHK2 IHC Staining (nuclear)
Case 1	frameshift/truncating	c.507delT	p.Phe169Leufs*2	Positive (>90%, mild-moderate)
Case 2	frameshift/truncating	c.1100delC	p.T367Mfs*15	Positive (>90%, strong)
Case 3	frameshift/truncating	c.1100delC	p.T367Mfs*15	Negative (No staining)
Case 4	missense	c.1283C>T	p.S428F	Positive (>90%, strong)
Case 5	missense	c.470T>C	p.I157T	Positive (>90%, strong)
Case 6	missense	c.1039G>A	p.D347N	Positive (>90%, strong)
Case 7	missense	c.470T>C	p. I157T	Positive (>90%, strong)
Case 8	missense	c.707T>C	p.Leu236Pro	Negative (No staining)

Figure 1 - 265

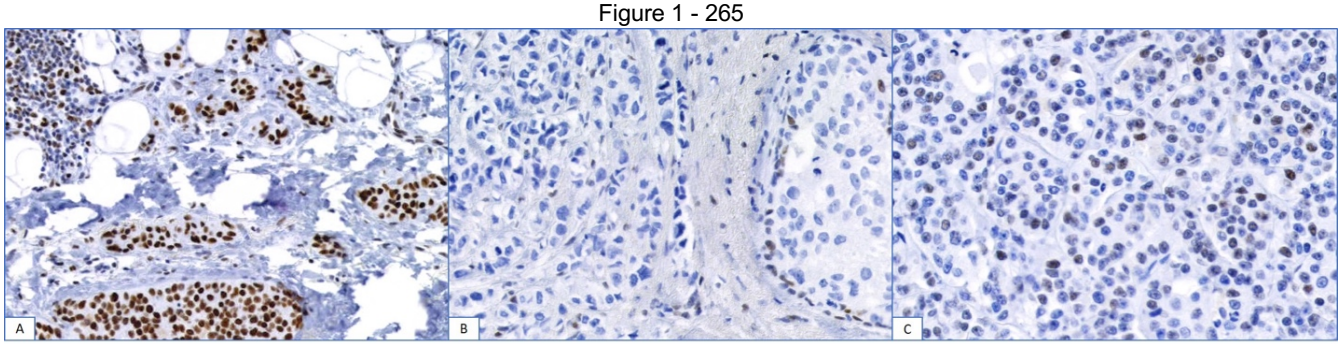
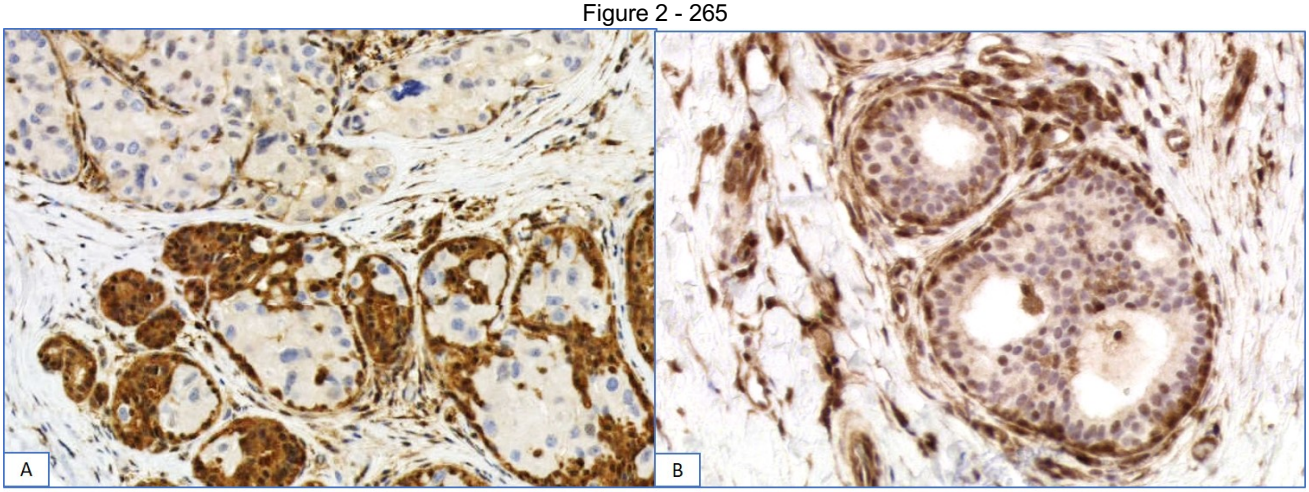


Figure 2 - 265



Conclusions: We report abnormal CHK2 IHC expression in 88% of BCs with germline *CHEK2* mutations. With *PTEN* germline mutations, IHC PTEN loss is seen as early as ADH, as well as in carcinoma. Abnormal CHK2 & PTEN IHC pattern can be used as a quick and cost-effective surrogate marker for identifying *CHEK2* & *PTEN* mutated BCs.

266 Reappraisal of Impact of Multiple Hematoxylin and Eosin Level Examination on Breast Cancer Sentinel Lymph Node Metastasis Detection and Pathologic Staging

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Background: Sentinel lymph node (SLN) excision is a standard procedure for surgical staging of early breast cancer (BC). Traditionally, histological evaluation of SLNs includes multiple H&E serial section examination, and keratin immunostaining. Impact of special histological processing of SLN on TNM staging has not been studied. Variation in metastatic tumor size and tumor foci in SLN level sections has not been studied systematically. Aim of this study is to analyze the impact of serial H&E examination of SLNs on metastatic tumor size measurements, pN staging and anatomic TNM staging of BC.

Design: Patients with BC who underwent primary breast surgery and SLN sampling during 2010-2015 were identified. Positive SLN that underwent H&E examination of 3 serial level sections and showed metastasis (mets) <20 mm were included in the study. Neoadjuvant cases were excluded. The SLN H&E slides were reviewed. SLN mets size and number of mets foci were recorded. Clinical, histological and follow up information was retrieved from cancer registry.

Results: Total 236 positive SLNs, from173 patients, were included in the study. There were 12 (5%) SLNs with isolated tumor cells, 73 (31%) with micromets and 151 (64%) with macromets. Multiple non-contiguous mets foci were identified in 30% SLNs. Mets were present in only 1 section in 23 (10%) SLNs, 2/3 sections in 30 (13%) SLNs and all 3 sections in 183 (77%) SLNs. Sections containing mets size of different pN categories were present in 84 (36%) SLNs. Ductal tumors showed significantly higher variation in mets size between levels than lobular tumors (40% versus 10%; p=.001). Variation in mets size did not correlate with presence of non-contiguous mulitple mets foci. Examining only first H&E section lowered pN staging in 30 (17%) patients and altered overall TNM staging in 27 (16%) patients. The most frequent alteration in pN staging with 1 H&E section was under staging of pNmi as pN0, in 18 (10%) patients. Using 2 H&E serial sections down staged pN and TNM staging in 7 (4%) patients (p<0.001; Vs. 1 H&E). Kaplan –Meier survival curves plotted similar progression free and overall survival estimates using AJCC staging with 2 and 3 H&E sections.

Conclusions: Examination of only 1 H&E section on axillary SLN misses up to 25% of microscopic metastasis and underestimates the anatomic AJCC staging in up to 16% breast cancer patients. Two H&E section evaluation performs almost as well as 3 H& E levels in identifying SLN metastases.

267 Molecular Profiling of Clear Cell Carcinoma of the Breast Reveals Novel Targetable Biomarkers

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Disclosures: Faruk Skenderi: None; Juan Palazzo: None; Jeffrey Swensen: *Employee*, Caris Life Sciences; Elma Contreras: None; Elena Florento: None; Zoran Gatalica: *Employee*, CARIS Life Sciences; Semir Vranic: None

Background: Although clear cell morphology may be seen in various subtypes of breast cancer, a pure variant of clear cell carcinoma (usually glycogen-rich) is a very rare primary breast malignancy. It is characterized by the neoplastic cells with abundant and clear cytoplasm that contains glycogen. A recent SEER study highlighted the aggressive clinical behavior of this rare cancer. Apart from steroid receptors profile (ER positive in 33-75%, PR positive in 30-43% and HER2 positive in 0-44%) status, no information is available regarding its molecular features and targetable biomarkers.

Design: Nine pure (>90% clear cell morphology) clear cell carcinomas of the breast were comprehensively profiled using massively parallel DNA and RNA sequencing (NGS), in-situ hybridization and immunohistochemistry.

Results: Steroid receptors ER and AR were positive in 8/9 and 7/9 cases, respectively. AR was positive in 6/7 cases without the presence of ARv7 splice variant. None of the cases was HER2 positive. Pathogenic mutations were found in *PIK3R1* and *BRCA2* (#1), *TP53, PTEN* and *CDKN2A* (#2), *TP53* and *BCOR1* (#3). PTEN protein loss was confirmed by IHC in *PTEN* mutated cases as well as in two additional cases without detectable *PTEN* gene mutation. No gene fusion was seen in any of the cases. Low PD-L1 expression (1-10%) was exclusively seen in immune cells (n=3/8). All tested cases (n=8) were MSI stable and had low TMB (3-7 mutations/mb) (n=3).

Conclusions: Clear cell carcinomas are predominantly ER and AR positive and consequently amenable for endocrine treatment regimes. Frequent AR over-expression without ARv7 may support trials with anti-AR therapies. A proportion of clear cell carcinomas harbors alterations in the PIK3CA/PTEN pathway indicating a potential benefit of PI3K/Akt/mTor inhibitors while the presence of PD-L1 on immune cells warrants the trials with immune checkpoint inhibitors.

268 Mammary Spindle Cell Proliferations on Core Needle Biopsy: Is Excision Always Necessary?

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Background: The category of mammary spindle cell proliferations (SCP) is heterogeneous and encompasses a wide range of lesions from benign to malignant. While historically most if not all SCP diagnosed on core needle biopsy (CNB) would be excised for definitive diagnosis, in modern era of minimally invasive treatment some of them may be followed with conservative approach. The aim of this study was to examine the spectrum of SCP and to evaluate if excision of benign / indeterminate SCP is required.

Design: We performed a retrospective review of all CNB with spindle cell proliferation or lesion or neoplasm or tumor diagnosis between 2001 and 2019. Clinical, pathological and follow up data were reviewed.

Results: We identified 71 CNB from 64 patients (5 with multiple biopsies of progressing/recurrent SCP; 3 male patients). Mean age was 52 years. Based on CNB 38 (53.5%) SCP were benign (differential diagnosis [ddx] included fibromatosis, myofibroblastoma, fat necrosis, schwannoma, leiomyoma, scar, hamartoma, benign fibroepithelial lesion, and/or reactive changes, regardless whether excision for definitive classification was recommended), 16 (22.5%) indeterminate (benign and malignant entities in ddx or excision for definitive classification recommended without ddx including benign entities only) and 17 (24%) were malignant (metaplastic carcinoma, borderline / malignant phyllodes or sarcoma favored). Clinico-radiologic findings and follow up (FU) are summarized in table 1. Benign SCP were more likely to occur in younger patients with no prior/concurrent breast cancer and be of smaller size. All male patients had benign SCP (schwannoma, myofibroblastoma, fibromatosis) that were not excised (FU 3-26 months). Among 18 benign SCP that were not excised 17 were stable, smaller or no longer present at median 42 months (range 6-193m; one lost to FU). All 3 indeterminate SCP on CNB that ended up being malignant on excision were highly suspicious radiologically (BIRADS at least 4c). All malignant SCP presented with either a new mass or within 6 months from the onset of symptoms.

CNB diagnosis	Benign n=38	Indeterminate n=16	Malignant n=17
Age (median, mean)	50, 48	51, 52	56, 60
Presentation	Palpable mass 24 (63%) Imaging 12 (32%) Pain 2 (5%)	Palpable mass 11 (69%) Imaging 5 (31%)	Palpable mass 10 (59%) Imaging 6 (34%) Pain 1 (7%)
Duration of symptoms	≤6months - 16 >6months - 8 Unknown - 13	≤6months - 9 >6months - 2 Unknown - 5	≤6months – 14 (2 with rapid growth in a known mass) >6months - 0 Unknown - 3
Size on imaging	Median 14 mm Mean 19 mm	Median 25 mm Mean 27 mm	Median 34 mm Mean 64 mm
Highly suspicious on imaging	Yes – 11 (31%) No - 24 Unknown - 3	Yes – 5 (33%) No - 10 Unknown - 1	Yes – 12 (80%) No – 3 Unknown - 2
Prior or concurrent breast cancer	9 (24%)	5 (31%)	8 (47%)
Diagnosis on excision	20 benign 18 not excised	13 (72%) benign 3 malignant (BoPT, metaplastic ca, sarcoma)	15 malignant (2 metastatic not excised)
Outcome (NED=No evidence of disease; AWD=Alive with disease; LFU=lost to follow-up)	22 NED 12 stable/smaller 4 LFU	14 NED 1 AWD 1 LFU	8 NED 7 AWD 2 LFU
FU in months (median, mean, range)	50, 65, 2-193	67, 67, 12-141	56, 60, 1-188

Conclusions: Most SCP were benign (72%). All malignant SCP were diagnostic on CNB. None of the benign SCP on CNB resulted in an upgrade to malignancy on excision. Indeterminate SCP on CNB are highly unlikely to be malignant if clinico-radiological correlation is ensured. Many SCP can be spared surgery provided good correlation with radiological and clinical data.

269 The Transcriptomic Landscape of Tumor-Adjacent Benign Breast in ER+ Breast Cancer

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Disclosures: Malvika Solanki: None; Asha Nair: None; Jaime Davila: None; Daniel Visscher: None; Jodi Carter: None

Background: The transcriptome of estrogen receptor (ER)-positive breast cancers (BC) has been widely studied. In contrast, tumor-adjacent benign breast is not well-characterized despite its frequent use as "normal" tissue. We compared the coding and long non-coding transcriptomes of "tumor-adjacent" benign breast to paired ER+ BC, and benign breast without BC, to gain insight into tumor-associated alterations in the benign microenvironment and at-risk benign breast.

Design: Methods: RNA from cryobanked, histologically-verified "tumor-adjacent" benign breast (benign $^{ER+BC)}$ with matched ER^+ BC (N=10), and benign breast from age-matched premenopausal patients with no BC (benign ^{NoBC}, N=10) was sequenced (Illumina TruSeq Stranded mRNA kit). Differential expression (DE) analysis (edgeR 2.6.2) identified DE genes from normalized RPKM reads (absolute log2 fold change (FC) > 1 and false discovery rate (FDR) < 0.10), with intra-group bias correction. Ingenuity pathway analysis (IPA), Ingenuity® Systems] and gene set enrichment analysis (GeneTrail) were performed.

Results: In benign $^{ER+BC}$ vs paired ER+BC, 2284 DE genes included coding RNAs (84%), antisense (AS) RNAs (4%) and lncRNAs (4%). In the benign $^{ER+BC}$ vs benign ^{NoBC} set, 484 DE genes included coding RNAs (84%), AS RNAs (2%) and lncRNAs (5%). Among 243 overlapping DE genes, the top RNAs included Kruppel-like factors (e.g. *KLF15, KLF2*), FAM proteins (*FAM107A; FAM13C*) and heat shock proteins (*HSPB6, HSPB7*) (p<0.001). Top DE genes up-regulated only in benign $^{ER+BC}$ included *PDK4, PER1, ZBTB16 KLF15, PCOLCE2,HIF3A, AQP7, LEP*, (p<0.001) Top canonical pathways altered in benign $^{ER+BC}$ vs. benign^{NoBC} included polo-like kinase signaling, CXCL signaling and cell cycle control/cyclin-dependent kinases (p<0.001). DE non-coding RNAs altered in benign $^{ER+BC}$ (N=30) included several lncRNA implicated in carcinogenesis or BC progression (e.g. AC083843, LINC00961,LINC001354 and RMRP).

Conclusions: Tumor-adjacent benign breast in ER+BC has coding and long noncoding RNA profiles that are distinct from both concurrent tumor and benign breast from age-matched patients without BC. We observed significant alterations in chemokine signaling, cell cycle control and many genes implicated in BC development and progression. These data provide insight into functional alterations of the peri-tumoral benign breast environment and at-risk benign breast.

270 Comparison and Assessment of Pathobiologic Indices to Predict Oncotype DX Recurrence Scores

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Disclosures: Thing Rinda Soong: None; Joseph Geradts: None

Background: The Oncotype DX Recurrence Score (RS) is used clinically to estimate the risk of distant recurrence for patients with ER-positive, lymph node-negative breast cancer and to help with decision-making about chemotherapy. One major drawback of the assay is its high cost. Composite indices based on tumor parameters have been proposed to be a no-cost alternative to the Oncotype DX assay. We aimed to validate the correlation of RS with 3 published pathobiologic composite indices that do not include Ki67 and to explore subset(s) of human breast carcinomas that may not need Oncotype Dx testing if RS can be predicted by other pathobiologic indices with high accuracy.

Design: Two hundred and twenty-nine breast cancer resections with data on RS were included (Table 1). Cases were reviewed for histology, overall grade, tubule score, nuclear grade, mitotic activity, and hormone receptor status. Pathobiologic predictive scores were calculated based on pathologic data and 3 published equations (Equation 1 per Klein et al 2013; Equation 2 per Geradts et al 2010; Equation 3 per Flanagan et al 2008)(Table 2; footnotes). Using RS as the gold standard, the predictive scores were evaluated for correlation and concordance with RS risk categories.

Results: All cases were ER-positive with 90% being PR-positive. The majority were moderately differentiated (69%) and of pT1 stage (70%). Based on RS, 62%, 31% and 7% of the cases were classified as having low, intermediate and high risk of recurrence respectively (Table 1). The 3 pathobiologic indices yielded comparable correlations with RS (Pearson correlation coefficients in overall population: Equation 1: 0.60; Equation 2: 0.59; Equation 3: 0.55). Equation 2 provided slightly better observed concordance with RS risk categories (66%) compared to equation 1 (concordance: 63%) and equation 3 (concordance: 59%), but the difference was not statistically significant (P=0.3). Agreement was best seen within the low-risk group, with equation 2 showing the greatest concordance (81%) (Table 2A). Among the 3 models, equation 1 achieved the highest specificity (84%) in distinguishing low-risk recurrence group from the rest of the cases (Table 2B).

Figure 1 - 270

Table 1. Tumor features in the study	
Characteristics	N=229 (Col %)*
Tumor size	Median: 1.6 cm; range: 0.3-10 cm
Tumor type	
Ductal	48%
Lobular	23%
Mixed ductal and lobular	27%
Others	2%
Overall tumor grade	
Grade 1	16%
Grade 2	69%
Grade 3	14%
Tumor pT stage	
1	70%
>1	30%
ER-positive	100%
PR-positive	90%
HER2 (immunohistochemical expression)	
Negative	92%
Equivocal	7%
Positive	1%
Oncotype DX Recurrence Score	
<18	62%
18-30	31%
>30	7%
*Column percentages may not add up to 100% due to missing data.	

Figure 2 - 270

Table 2. Concordance between Oncotype DX Recurrence Scores and predictive scores generated by 3 pathobiologic indices

A

		Oncotype DX Recurrence Scores (RS) N (%)			
Pathology-generated equations*	**Predicted scores**	**<18 (low risk)**	**18-30 (intermediate risk)**	**>30 (high risk)**	
Equation 1 (Klein et al 2013)	**<18**	90 (64)	16 (22)	1 (6)	
	18-30	51 (36)	50 (69)	11 (69)	
	>30	0 (0)	11 (9)	4 (25)	**Concordance=63%**
Equation 2 (Geradts et al 2010)	**<18**	112 (81)	28 (39)	2 (12)	
	18-30	22 (16)	31 (44)	6 (38)	
	>30	5 (3)	12 (17)	8 (50)	**Concordance=66%**
Equation 3 (Flanagan et al 2008)	**<18**	106 (75)	38 (53)	3 (19)	
	18-30	32 (23)	24 (33)	8 (50)	
	>30	3 (2)	10 (14)	5 (31)	**Concordance=59%**

B

	Distinguishing low-risk recurrence group from intermediate or high-risk recurrence groups based on RS	
	Sensitivity (%)	**Specificity (%)**
Equation 1 (Klein et al 2013)	58	84
Equation 2 (Geradts et al 2010)	67	79
Equation 3 (Flanagan et al 2008)	57	72

***Pathology-generated equations**

Equation 1 (Klein et al; Magee equation 2)
18.8042 + Nottingham score*2.34123 + ER IHC score*(- 0.03749) + PR IHC score*(- 0.03065)+ (HER2 score) + tumor size*0.04267
IHC score: negative: 0; positive: 1
HER2 score: negative:0; equivocal: 1.82921; positive: 11.51378

Equation 2 (Geradts et al)
40.0 -5.3*(ER Allred score) -2.7*(PR Allred score) + 13.0*(HER2 score) + 2.3*(tubule score) + 2.4*(nuclear grade score) + 6.5*(mitotic activity score)
HER2 score: negative:1; equivocal: 2; positive: 3

Equation 3 (Flanagan et al)
13.424+5.420*(nuclear grade score) + 5.538*(mitotic activity score)- 0.045*(ER H score) - 0.030*(PR H score) + 9.486*(HER-2/neu score)
HER-2/neu score: negative: 0; equivocal: 0.5; positive: 1
HER-2/neu was considered positive with either 3+ immunoreactivity or amplification by FISH

Conclusions: The 3 pathobiologic indices demonstrate comparable performance in predicting RS, with equation 2 showing slightly higher observed categorical concordance. The indices are better applied to predicting low-risk status but misclassification ranging from 20-30% for low-risk cases can occur as observed in our cohort.

271 Predicting Recurrence and Chemotherapy Benefit for Breast Cancer Patients Using Deep Learning Models of Histology Images

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Disclosures: Arunima Srivastava: None; Asmaa Aljuhani: None; Vidya Arole: None; Satoshi Hamasaki: None; Zaibo Li: None; Raghu Machiraju: None; Anil Parwani: None

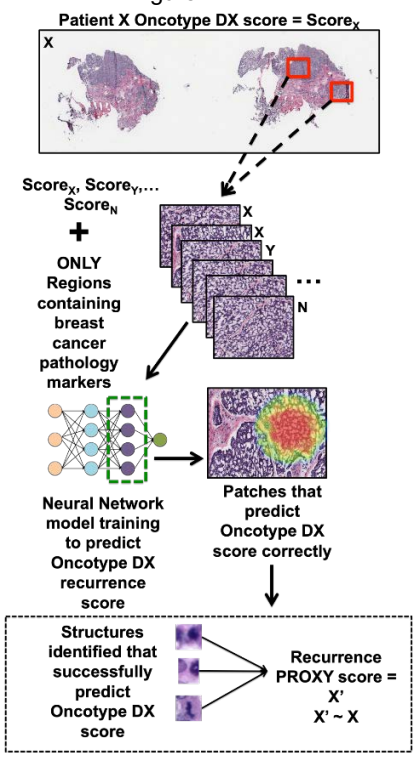
Background: The Oncotype DX assay assesses the expression of 21 genes to generate a recurrence score between 0-100, predicting possibility of recurrence and consequently, chemotherapy benefit in patients with estrogen receptor-positive invasive breast carcinoma. Patients with low recurrence scores (<18) do not benefit from chemotherapy whereas patients with high scores (>30) do. However, Oncotype DX is an expensive test with a cost of about 4000$. This work aims at building a deep learning model with histology images to identify pathology structures that may serve as a proxy for the Oncotype DX recurrence score, hence lowering the cost of predicting recurrence risk and chemotherapy benefit.

Design: A dataset of 94 breast invasive carcinoma cases (288 whole slide images) with available Oncotype DX scores was utilized to build a recurrence prediction model using a neural network, inspired by the existing state-of-the-art VGG19 architecture. This model was trained to focus on regions relevant to breast carcinoma histologic grade by automatically evaluating mitotic activity, tubule formation and nuclear grade, which are the main pathology indicators used to diagnose grade of breast cancer via the Nottingham score. We visualized the various layers of the trained neural network to identify structures and areas of interest to the model. With the aid of subject matter experts and existing literature, we can associate the now identified structures to the gene set for Oncotype DX and build a histological marker proxy for the Oncotype DX.

Results: The current model is successfully able to classify tumor cells, mitotic cells and tubule formation within a test compendium of histology image patches (>90%, >80% and >70% accuracy respectively). Additionally, a framework to visualize the regions of interest to the neural network has been built and validated by pathologists. Currently scanning of tissue blocks and annotation of whole slide histology images are underway for model training and recurrence score prediction.

	Accuracy	Sensitivity	Specificity
Tumor Cell	96%	96%	97%
Mitotic Cell	81%	71%	91%
Tubules	70%	65%	75%

Figure 1 - 271



Conclusions: In summary, we have developed a deep learning model to grade breast carcinoma histologically by automatically evaluating several histological parameters, which can be used to build a recurrence prediction model to serve as a proxy for Oncotype DX assay. A successful predictive model will not only address the need for a cost-effective solution for assessing chemotherapy benefit to patients, it will further establish understanding between genetic and histological biomarkers.

272 Diagnosing Breast Cancer Brain Metastases: What Immunohistochemical Panel Should I Choose?

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Disclosures: Eric Statz: None; Julie Jorns: None

Background: Following lung cancer, breast cancer is the second most common metastatic tumor to the brain, of which triple negative breast cancers (TNBC) and HER2+ breast cancers are the most common subtypes. TNBC do not have standard immunoprofiles and can be difficult to distinguish from brain metastases from other primaries. A recent paper found SOX-10 to be a sensitive and specific marker for TNBC in a differential with TTF-1 negative lung adenocarcinoma. In light of this new data, we sought to identify the optimal panel for diagnosis.

Design: Clinicopathologic features including subtype, grade, biomarker status, presence of other distant metastases, genetic risk, age at original and brain metastasis diagnoses, and living/deceased status were assessed by chart and slide review.

A tissue microarray (TMA) was created from 47 female patients with breast cancer metastases to the brain and 15 paired breast primaries. Original blocks were used if TMA material had inadequate tumor for interpretation. Immunohistochemistry was performed for CK7, GATA3, SOX-10, Mammaglobin, and GCDFP-15. A result was considered positive if at least moderate staining was seen in ≥1% of tumor cells.

Results: Patients were predominantly Caucasian (38/47, 80.9%) with mean age at diagnosis of 48.8 yrs (range 22-77). Most were grade 3 (42/47, 89.4%) and ductal (40/47, 85.1%). Most (38/47, 80.9%) had died of disease, with mean time to death of 1.5 yrs (range 0.2-5.6).

Of brain metastases, 24 (51.1%) were HER2+, 14 (29.8%) TNBC and 9 (19.1%) luminal. Forty-five (95.7%) were CK7 positive, 36 (76.6%) GATA3 positive, 7 (14.9%) SOX-10 positive, 20 (42.6%) mammaglobin positive and 19 (40.4%) GCDFP-15 positive. At least one of CK7, GATA3, or SOX-10 was positive in all TNBC metastases. Amongst the 7 SOX-10+ cases, CK7 was positive in 6, and GATA3 was positive in one and rare weak in another. Figure 1 highlights three representative cases.

Of the 15 cases with paired breast primaries there was relatively good IHC correlation, with highest variability in mammaglobin expression (3/15, 20%) between metastatic and primary tumor.

SOX-10 was positive in background glial cells as seen in Figures 1 and 2, occasionally complicating interpretation.

Breast Cancer Brain Metastasis IHC Expression by Subtype

	TNBC (N=15)	Luminal (N= 9)	HER2+ (N=23)
CK7	14 (93%)	8 (89%)	23 (100%)
GATA-3	8 (53%)	8 (89%)	20 (87%)
SOX-10	6 (40%)	0 (0%)	1 (4%)
Mammaglobin	2 (13%)	6 (67%)	12 (52%)
GCDFP-15	3 (20%)	4 (44%)	12 (52%)

Figure 1 - 272

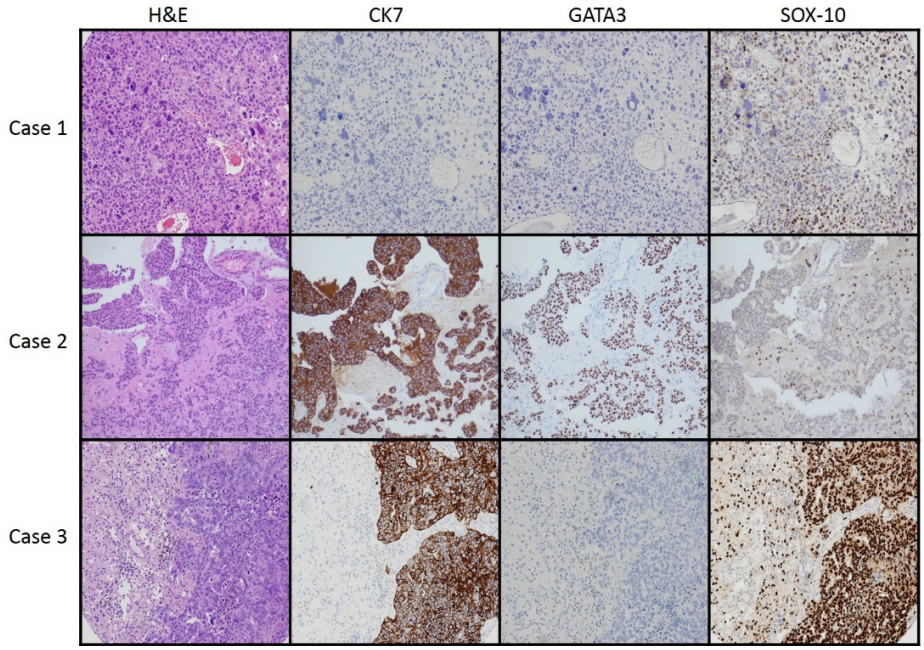
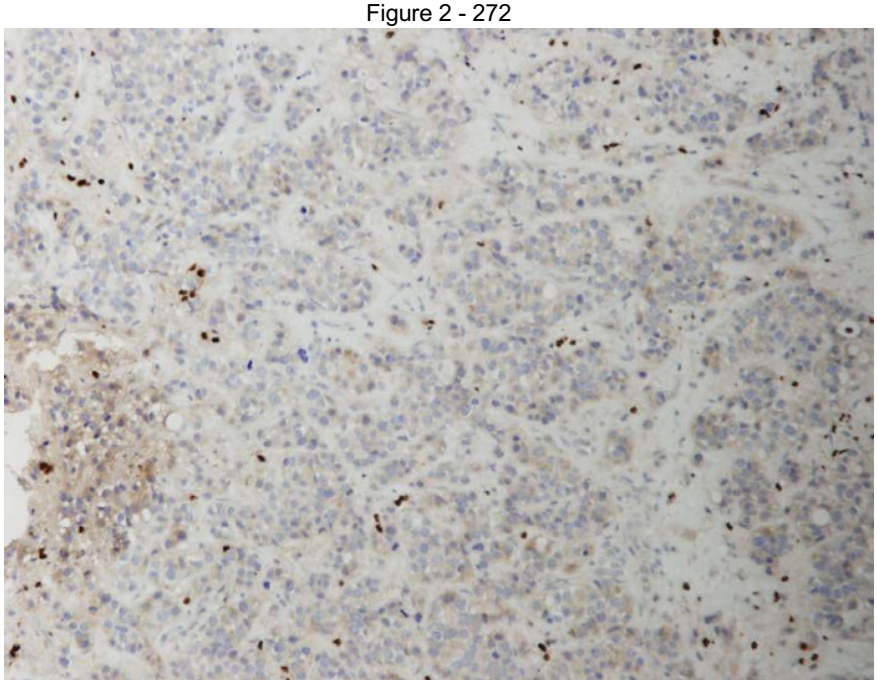


Figure 2 - 272



Conclusions: A panel of CK7, GATA3 and SOX-10 appears complementary in the diagnosis of breast cancer brain metastasis as at least one marker was positive in all cases. SOX-10 appears to be specific, but, like mammaglobin and GCDFP-15, is not a particularly sensitive marker in this context.

273 A Novel Case of Mammary-Type Myofibroblastoma with Sarcomatous Features and Underlying Identical MET Mutations

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Disclosures: Alexander Strait: None; Konstantinos Linos: None; Kristen Muller: None

Background: Myofibroblastoma (MFB) is a rare benign spindle cell tumor of the breast and soft tissue exhibiting a wide morphologic spectrum. The majority of these tumors harbor 13q14 deletions leading to loss of Rb1 protein expression, a feature shared among several soft tissue tumors with morphologic similarities (i.e., spindle cell lipoma and cellular angiofibroma). Recently, cellular angiofibroma with atypia and sarcomatous transformation has been described; despite the alarming histology, these tumors appear to follow an indolent course. Although degenerative-type atypia can occur in MFB, sarcomatous-like transformation has never been described.

Design: We present a novel case of a 70-year-old man who underwent excision of a 2.3 cm left breast mass. Microscopic and immunohistochemical analysis revealed a MFB with distinct areas of sarcomatous-like morphology (Fig1A). Two expert soft tissue pathologists reviewed the case and confirmed the diagnosis. Both regions of classic MFB and sarcomatous-like areas were dissected and subjected to next-generation sequencing (NGS) and single nucleotide polymorphism-based chromosomal microarray analysis (CMA).

Results: Microscopically, the tumor was comprised of uniform spindle cells with fascicular architecture, associated stromal ropey collagen bundles and inconspicuous mitotic activity (Fig1B). There was an abrupt transition to a morphologically distinct component with sarcomatous morphology in which tumor cells exhibited significant pleomorphism and increased mitotic activity (Fig1C). By immunohistochemistry, both components were diffusely positive for smooth muscle actin, desmin, progesterone receptor, and BCL2, whereas both showed loss of Rb1 expression. CD34 was positive in the classic MFB, but negative in sarcomatous-like cells. In addition, the sarcomatous-like component showed an overexpression of p16 and absence of p53 by IHC. CMA revealed similar copy number alterations in both profiles including a single copy loss of 13q14 (Fig2). The sarcomatous morphology had an additional loss of chromosome 17p. Both components showed identical *MET* mutations (c.504G>T, p.E168D); an additional *TP53* deletion (c.836_861del, p.G279fs) was found in the sarcomatous component. NGS did not reveal mutations in the *RB1* gene, despite an absence of Rb1 protein expression, possibly due to a SNV or point mutation not covered by our hotspot NGS panel.

Figure 1 - 273

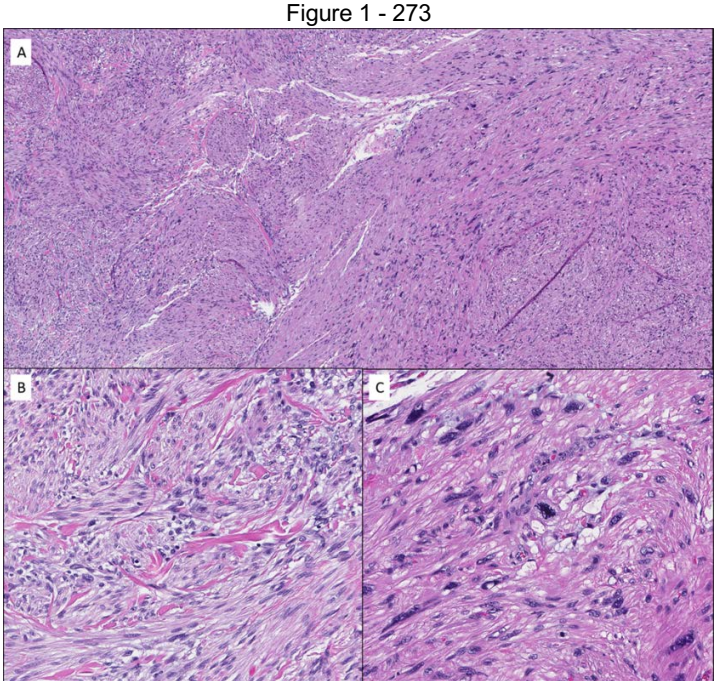
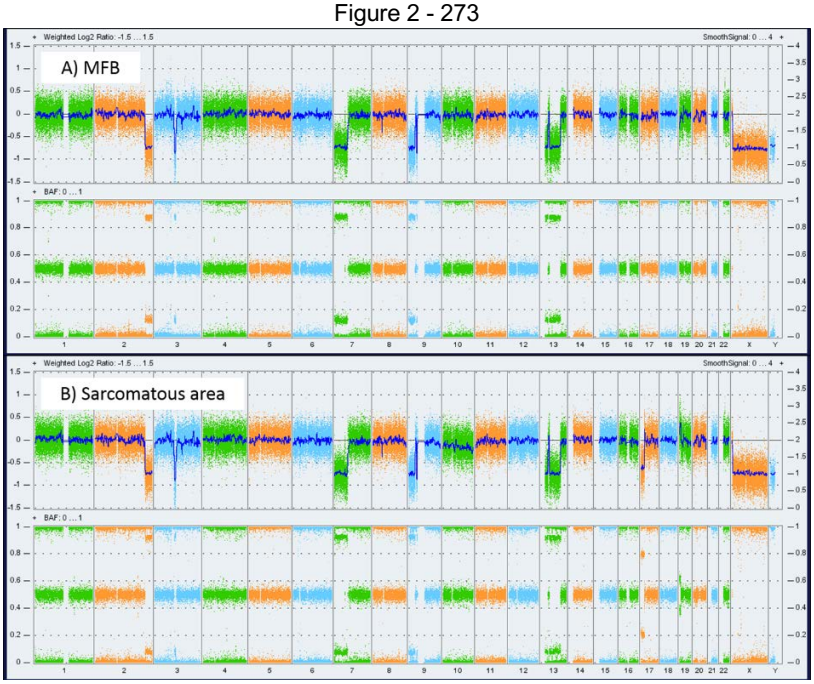


Figure 2 - 273



Conclusions: To our knowledge, this represents the first reported case of sarcomatous-like transformation arising in a MFB.

274 Genetic Alterations Between Micropapillary Variant of Mucinous Carcinoma (MPMC) and Conventional Pure Mucinous Carcinoma (cPMC) of Breast

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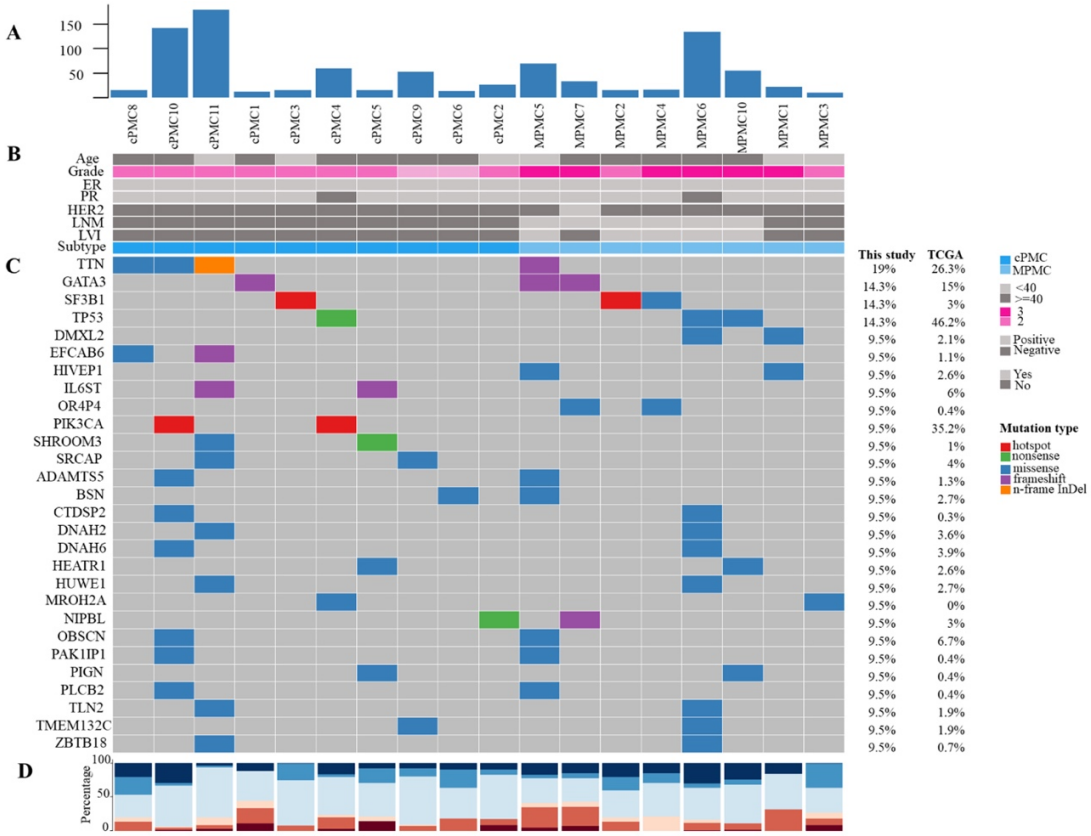
Disclosures: Peng Sun: None; Jiehua He: None; Zaixuan Zhong: None; Xue Chao: None; Mei Li: None; Rongzhen Luo: None; Rongzhen Luo: None; Rongzhen Luo: None; Dan Chen: None

Background: Pure mucinous carcinoma (PMC) is a rare histologic form of breast carcinoma characterized by clusters of tumor cells floating in large amounts of extracellular mucin. Micropapillary pattern (MP) may occur in PMC named micropapillary variant of mucinous carcinoma (MPMC), which may associate with a higher rate of nodal metastasis and lymphovascular invasion than conventional PMC (cPMC). Increasing studies have been conducted to characterize the difference of morphology and prognostic significance between MPMCs and cPMCs, however, whether they harbored specific or overlapping genomic alteration has yet to be characterized.

Design: FFPE samples of tumors and adjacent normal tissues from 10 MPMCs (MP%>50%) and 11 cPMC were subjected to whole-exome sequencing with a coverage of 200 ×. Somatic mutations, copy number alterations and mutational signatures were determined with strictly integrated bioinformatics workflow.

Results: We identified 938 somatic mutations including 782 missense, 81 frameshift, 49 nonsense and 26 in-frame InDel in 21 cases of PMC. TTN (19.1%, 4/21), GATA3 (14.3%, 3/21), SF3B1 (14.3%, 3/21) and TP53 (14.3%, 3/21) were the most frequently mutated genes. Notably, PMCs tend to display a significantly lower mutation frequency of TP53 (14.3% vs. 46.2%, p=0.003) and PIK3CA (9.5% vs. 35.2%, p=0.017) than invasive ductal carcinomas (IDCs, NOS) from TCGA. Moreover, MPMC and cPMC harbored specific genomic alterations, in which PIK3CA (18.2%) were frequently found in cPMC, while GATA-3(20%), TP53 (20%) and SF3B1 (20%) recurrently mutated in MPMC. PIK3CA hotpot mutations (E545K, M1043I) were exclusively detected in cPMCs. The signature 3 (homologous recombination) and signature 13 (AID/APOBEC family) were prominent in MPMCs. Signature 1 and 2 displayed a dominate which have been linked to aging and activity of the AID/APOBEC family were identified in cPMCs. Copy number variation analysis revealed that MPMCs harbored significant arm-level alterations including chromosomal gains at 8q, 17q and 20q, as well as chromosomal loss at 6q, 17p. Meanwhile, significant gains at 6p, 8q as well as deleted regions at 6q were examined in cPMC. Besides, PI3K-Akt pathway, mTOR signaling pathway and AMPK signaling pathway were more recurrently deregulated in MPMCs than in cPMCs.

Figure 1 - 274



Conclusions: PMCs show a pattern of somatic genetic alterations different from IDCs dramatically. MPMCs, which may be noted as a distinct subtype of breast carcinoma, also harbored unique genetic alterations other than PMCs.

275 Invasive Apocrine Carcinoma of the Breast: Clinicopathologic Features and Comprehensive Genomic Profiling of 18 Pure Triple Negative Apocrine Carcinomas

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Disclosures: Xiangjie Sun: None; Ke Zuo: None; Wentao Yang: None

Background: Invasive apocrine carcinoma (IAC) is a rare type of primary breast cancer, constituting approximately 1% of all breast cancers. Most pure IACs are triple negative, while the rest are HER2-enriched. The lack of targeted therapies with TNBC has fostered efforts to discover actionable molecular targets in these tumors. To this end, we analyzed the clinicopathologic characteristics and comprehensive genomic profiling (CGP) in 18 pure triple negative apocrine carcinomas (TNACs) to elucidate the potentially actionable genomic alterations in this population.

Design: 18 pure TNAC patients who underwent breast surgery at Fudan University Shanghai Cancer Center were collected. Clinicopathologic characteristics were analyzed. All 18 specimens were sequenced by DIAN (Hangzhou Lab) using a 324-gene platform (FoundationOne CDx) with licensed technologies. In this study, we focused on clinical relevant genomic alterations (CRGAs) which were known or likely pathogenic alterations.

Results: The median age of these TNACs was 55.5 years, and postmenopausal status ratio was 77.8%. 83.3% of cases were diagnosed as histological grade II, and 16.7% as grade III. 50% of patients had nodal metastases at diagnosis. The majority of patients presented at low stage (I:38.9%; II:50.0%; and III:11.1%). Mean Ki-67 index was 9.7%. All cases exhibited diffusely nuclear staining for AR. PD-L1 positivity was 11.7%. With a median follow-up period of 76.5 months, one patient died and two experienced distant metastases. There were 61 CRGAs for all 18 pure TNACs, and mean tumor mutation burden was 3 Muts/Mb. The top ranked altered genes were PIK3CA (72%), PTEN (33%) and TP53 (28%). All the mutations in PIK3CA were known pathogenic mutations, while three novel frameshift mutations found in PTEN have not been reported before. Moreover, an actionable rearrangement involving the FGFR2-TACC2 was detected, which has not

been reported in breast cancer. In total, 88.9%, 50%, 44.4% and 16.7% of TNACs had at least one CRGA in PI3K/mTOR, cell cycle, RAS/RAF/MEK and growth factor receptors related pathways, respectively (Fig.1). All cases had at least one CRGA and 94.4% had at least one actionable alteration.

Figure 1 - 275

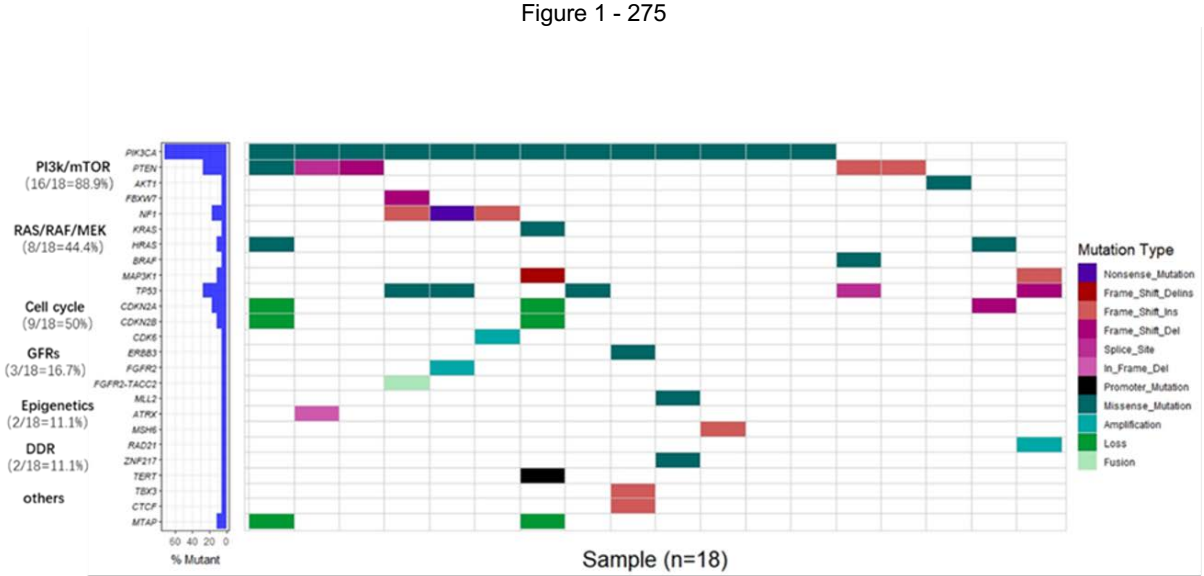

Figure 1. The landscape of somatic genetic alterations in 18 TNACs. In total, 88.9%, 50%, 44.4% and 16.7% of TNACs had at least one CRGA in the PI3K/mTOR, cell cycle, RAS/RAF/MEK and growth factor receptors (GFRs) signaling pathways, respectively.

Conclusions: To the best of our knowledge, this study is the largest sequenced cohort for pure TNACs. We demonstrated that most pure TNACs had at least one actionable alteration. Incorporating CGP into TNACs might shed light on the potential therapeutic opportunities for both targeted drugs and immunological checkpoint inhibitors.

276 Characterizing PD-L1 Expressing Immune Cells in Triple Negative Breast Cancer by Multiplex Immunofluorescence

Xiangjie Sun[1], Edwin Parra[1], Mei Jiang[1], Luisa Solis[1], Aysegul Sahin[1], Ignacio Wistuba[1], Jennifer Litton[1], Fei Yang[1]
[1]The University of Texas MD Anderson Cancer Center, Houston, TX

Disclosures: Xiangjie Sun: None; Edwin Parra: None; Mei Jiang: None; Luisa Solis: None; Aysegul Sahin: None; Ignacio Wistuba: None; Jennifer Litton: *Primary Investigator*, Pfizer/Medivation; *Grant or Research Support*, EMD Serono; *Grant or Research Support*, Genentech; *Primary Investigator*, Astra Zeneca; Fei Yang: None

Background: Tumor infiltrating lymphocytes (TILs) has become a topic of interest as a prognostic and predictive biomarker in triple negative breast cancer (TNBC). Immunotherapy is a rapidly evolving field for the treatments on TNBC. In the IMpassion130 Trial, advanced TNBC patients treated with Atezolizumab plus nab-Paclitaxel had a better PRS and OS compared with patients treated by nab-Paclitaxel if the tumor infiltrating immune cells (ICs) were PD-L1 positive. To improve the understanding of tumor immune microenvironment, we investigated PD-L1 expressing tumor infiltrating ICs and phenotyping of TILs in TNBC by multiplex immunofluorescence (mIF).

Design: We studied 157 surgical resected breast cancer tissue placed in a tissue microarray with three 1-mm^2 cores per tumor, including 102 TNBCs (ER/PR <1%), 41 ER/PR low expression cases (ER/PR 1~9%), and 14 luminal types as control. The FFPE sections were stained by mIF for two panels, including Panel 1 (PD-L1, PD-1, CD3, CD8, CD68 and panCK) and Panel 2 (FOXP3, Granzyme B, CD45RO, CD3, CD8 and panCK), using Opal™ 7-color Kit on Leica BOND RX. After scanning in the Vectra system (Akoya/Perkin Elmer), PD-L1 expressing ICs and other immune phenotypes were analyzed via InForm software (Akoya/Perkin Elmer).

Results: PD-L1 expression was firstly evaluated using a standard microscopy approach. PD-L1 positive was defined as either ≥1% of malignant cells with membrane staining or ≥1% of ICs (Fig 1). PD-L1 protein was expressed in 35.3% (36/102) of TNBCs and 29.3% (12/41) of ER/PR low expression cases. Only 14.3% (2/14) luminal type cases were PD-L1 positive. After co-localizing PD-L1 with other immune markers, PD-L1 expressing ICs were identified, including PD-L1+ macrophages and PD-L1+ lymphocytes (Fig 2A). We are quantifying the cell subtypes and will provide density and/or percentage of the immune cells based on co-localization of all markers. It

includes macrophages PD-L1+ (CD68+/PD-L1+), cytotoxic T cells PD-L1+ (CD3+/CD8+/PD-L1+), antigen-experienced T cells (CD3+/PD-1+), activated cytotoxic T cells (CD3+/CD8+/GranzymeB+), memory/regulatory T cells (CD3+/CD45RO+/FoxP3+), and so on (Fig 2A/B). Spatial distribution of the phenotypes will be generated as well.

Figure 1 - 276

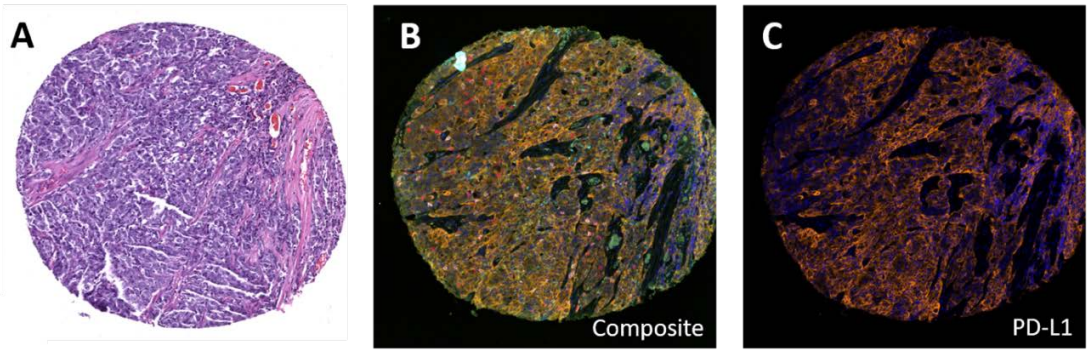

Figure 1. Representative images of one TNBC core, including H&E (A), composite image of panel 1 (B) and PD-L1 single marker image (C).

Figure 2 - 276

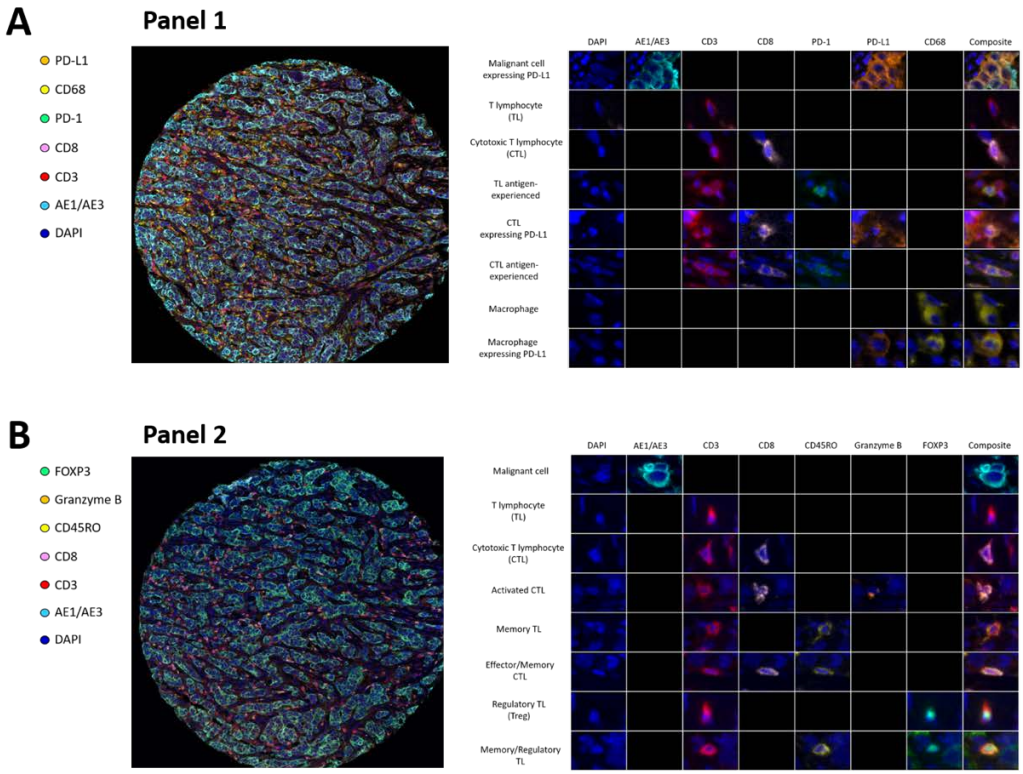

Figure 2. Multiplex IF composite images and immune cell phenotypes in panel 1 (A) and panel 2 (B).

Conclusions: Phenotyping tumor immune cells in the study is highly informative to understand the immune microenvironment in TNBC. Characterizing PD-L1 expressing immune cells in TNBC might be helpful to predict the immunotherapy of PD-L1/PD-1 blockade.

277 Trucut Biopsy and F N A C for Breast Lumps: Why Can't Pathologist Perform Both with Improved Results?

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Disclosures: Bindu TG: None; Unni Pillai: None

Background: Triple assessment comprising of clinical examination,mammogram and pathological analysis is the gold standard in diagnosis of any breast swelling. Pathological evaluation includes Fine Needle Aspiration Cytology(FNAC) or Trucut Biopsy;FNAC is usually performed by pathologists with far superior yield, while Trucut Biopsy is the domain of Interventional Radiologist and Surgeon. Trucut biopsy has distinct advantage over FNAC in assessment of tumor grade, hormonal evaluation and other ancillary studies. A skilled Pathologist can perform Trucut Biopsy sans difficulty with added advantage of Rapid Onsite Evaluation(ROSE) to confirm adequacy of material.

Design: 154 patients between August 2017 and December 2018, attended Surgery OP with palpable breast lumps, radiologically suspicious of malignancy (BIRADS IV and V) were included in the study. Both FNAC and Trucut biopsy were done by the same operator (First Author) at a single sitting. Location of the tumor and tumor heterogenisity were assessed correlating clinical and Radiological features. Minimum of 3 smears and clot for proceing were obtained after FNAC (Using 24 G needle and 10 ml syringe) and 4-6 tissue cores of minimum 1 cm long by Trucut biopsy (using 14 G needle) for Hematoxylin&Eosin(H&E) staining, Receptor study and molecular analysis.

Results: Trucut biopsy was positive in 146 cases and negative in 8 cases. Among the negative cases,3 were positive on FNAC. Remaining 5 patients needed image guided biopsy. FNAC was positive in 127 cases and negative in 27 cases. Negative cases could be corelated with Trucut Biopsy. Trucut Biopsy was less productive in skin and chest wall adherant tumors and schirrous tumors within loose breast tissue where FNAC fared better. In the present study Trucut Biopsy had a sensitivity of 94% and specificity of 100%.FNAC had a sensitivity of 82%.All the 154 cases had Excision Biopsy. 123 cases had primary surgery and 31 had Neoajduvant Chemotherapy.

Conclusions: Trucut Biopsy is the better tool for the initial assessment of palpable breast lump. It is an easy and simple technique which can be mastered by a Pathologist who have adequate skill to perform FNAC. The advantage of Pathologist is that, better assessment of adequacy of the material, by naked eye examination of the tissue cores and Rapid On Site Evaluation by imprint smears or crush preparation, which will improve the patient management.

278 Determination of HER2 Status in Breast Carcinoma Using Droplet Digital PCR: An Analysis of Concordance between Standard Methods and Four Unique Reference Probes

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Disclosures: William Towne: None; Yuewei Li: None; Swarna Gogineni: None; Susan Mathew: None; Paula Ginter: None; Wei Song: None

Background: Human epidermal growth factor receptor 2 (HER2) is a predictive and prognostic marker in breast cancer, and it is routinely assessed by immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH). Droplet digital PCR (ddPCR), a highly accurate method to quantify DNA copy number, is potentially a robust alternative for HER2 diagnostics. When compared to HER2 IHC and FISH testing ddPCR shows 79.3% to 100% sensitivity and 88.5% to 100% specificity. Most importantly, ddPCR allows interrogation of multiple regions on chromosome 17 as references for HER2 quantification. Given this advantage, ddPCR may be a better choice to detect HER2 copy number changes in cases with polysomy, which present a constant challenge in HER2 FISH testing. We sought to determine the concordance of ddPCR with IHC and FISH testing at our institution.

Design: ddPCR was performed on formalin-fixed paraffin-embedded tissue from 35 cases of invasive breast carcinoma. HER2 IHC status for all cases was based on the 2013 and/or 2018 ASCO/CAP HER2 guidelines and were as follows: 9 positive, 9 negative, and 17 equivocal (2+). Of IHC equivocal cases: 6 were FISH positive, 7 were negative, and 4 were equivocal by 2013 guidelines. By the 2018 guidelines, all four equivocal cases were negative. ddPCR involved a probe-based PCR reaction mixture comparing the ERBB2 gene with four unique reference gene probes on chromosome 17 (*RARA*, *TP53*, *YWHAR*, and *B3GNTL1*). A cutoff of 4.0 HER2 copy number variants (CNV) was used to determine positivity. Clinical-pathologic characteristics were also examined.

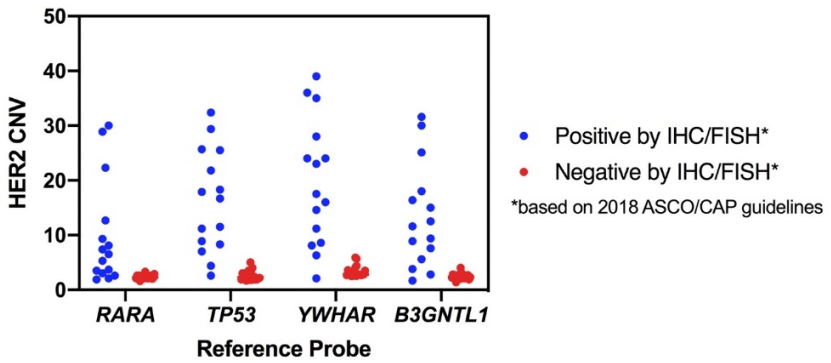
Results: Of the four reference probes examined, all probes showed good (≥80%) sensitivity and specificity, with the exception of *RARA* which showed a sensitivity of 60% (**Table 1**). When the results from the other three probes (*TP53*, *YWHAR*, and *B3GNTL1*) were analyzed in combination, HER2 amplification was concordant in all but one case (34/35; 97.1%). This case was 3+ by IHC and negative by ddPCR. HER2 FISH was subsequently performed on this case and was negative, suggesting an alternative mechanism of HER2 protein overexpression.

Table 1. Sensitivity and Specificity of Four ddPCR Reference Probes

Reference Probe	HER2 Status by ddPCR	Positive by IHC/FISH*	Negative by IHC/FISH*	Sensitivity	Specificity
TP53	Positive	14	2	93%	90%
	Negative	1	18		
B3GNTL1	Positive	12	1	80%	95%
	Negative	3	19		
YWHAR	Positive	14	4	93%	80%
	Negative	1	16		
RARA	Positive	9	0	60%	100%
	Negative	6	20		
*based on 2018 ASCO/CAP guidelines					

Figure 1 - 278

Figure 1. HER2 CNV of 35 breast carcinoma cases determined by ddPCR with four reference probes.



Conclusions: Concordance of ddPCR with standard methods for determination of HER2 status was excellent. The genes *YWHAR*, *TP53*, and *B3GNTL1* were all good reference probes, particularly when analyzed together. ddPCR using a combination of reference probes may be a useful addition or alternative method for determination of HER2 status in breast cancer.

279 Prognostic Value of Size, Node, and Ki-67 index (SiNK) in Breast Cancer

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Disclosures: Swikrity U Baskota: None; Rohit Bhargava: None

Background: SiNK index is a simple mathematical formula devised at our institution during the evaluation of Prosigna® breast cancer assay and its correlation to tumor clinical-pathologic features. The index is calculated by adding tumor **Si**ze (in mm) to the pathologic **N**odal stage (N-stage multiplied by a factor of 10) plus the **Ki**-67 labeling index. The score is categorized similar to Prosigna scores and risk categories. For lymph node-negative cases, the risk categories are low-risk (score 0-40), intermediate-risk (scores 41-60), and high-risk of recurrence (scores 61 or more). For lymph node-positive cases, the risk categories are low-risk (score 0-40) and high risk (score 41 or more). During index development, the concordance between Prosigna and SiNK score categories was 70% and the concordance between Prosigna risk categories and SiNK risk categories was 77%.

Design: This study was undertaken to further evaluate the clinical significance of SiNK index. A well-characterized in-house data set of 121 ER+/HER2-negative invasive breast carcinomas with relatively long-term follow up (average follow up of 98 months) was used for validation. SiNK indices were calculated and divided into scores 0-40, scores 41-60, and scores 61 or higher. The low, intermediate and high-risk categories were assigned based on lymph node status. Kaplan-Meier survival curves for disease-free survival (DFS) and overall survival (OS) were analyzed with respect to SiNK risk categories. *P*-values were obtained using the log-rank test.

Results: The recurrence rate was 5%, 11%, 33% for low, intermediate, and high-risk SiNK categories respectively. The overall survival was 67-71% for intermediate/high-risk SiNK categories and 89% for low-risk SiNK category (see figure 1).

Patient and tumor characteristics:	
Age in years	Mean: 59.9; Median 59; Range: 38-90
Size in mm	Mean: 16; Median: 15; Range: 6-73
Grade	I: 40 (33%); II: 56 (46%); III: 25 (21%)
Tumor type	Ductal: 110 (91%); Lobular: 9 (7%); Mixed: 2 (2%)
Lymph node status	Negative: 76 (63%); Positive: 45 (37%)
AJCC Stage	I: 75 (62%); II: 31 (26%); III: 15 (12%)
Ki-67 labeling index	Mean: 18.7; Median: 13; Range: 1-86
PR Status	Negative: 11 (9%); Positive: 110 (91%)
Systemic therapy	Chemo only: 2 (1.6%); Chemo+endo: 48 (39.6%) Endo only: 42 (34.7%) None: 8 (6.6%); Unknown: 21 (17.3%)

Figure 1 - 279

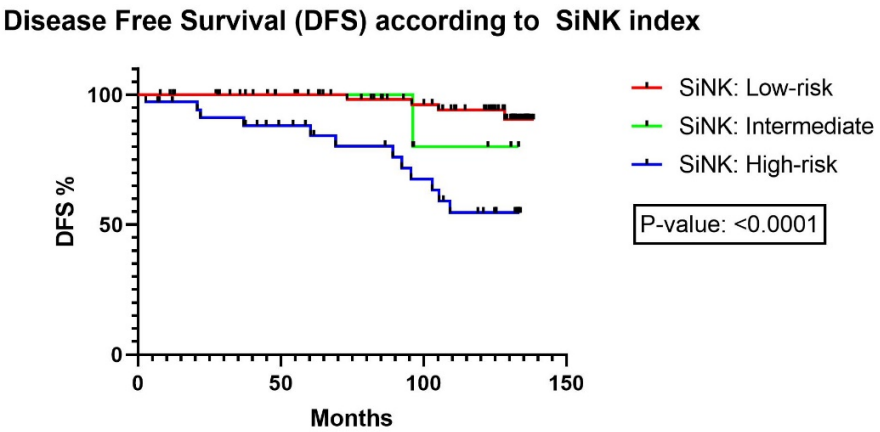
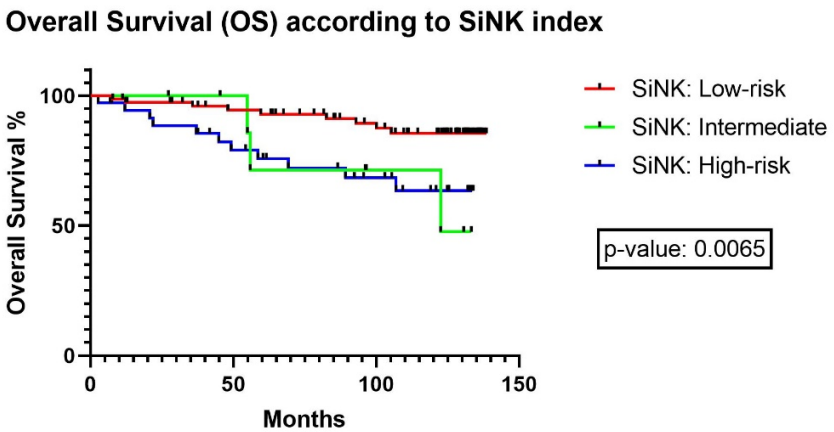


Figure 2 - 279



Conclusions: The SiNK index provides prognostic information comparable to expensive molecular assays. SiNK index is simple to calculate using data from routine pathology reports.

280 Genetic Alterations Targeting KIT in Breast Cancer

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Disclosures: Mahsa Vahdatinia: None; Ferrando Lorenzo: None; Dara Ross: None; Ahmet Zehir: *Speaker*, Illumina; Hannah Wen: None; Edi Brogi: None; Britta Weigelt: None; Sarat Chandarlapaty: *Consultant*, BMS; *Consultant*, Novartis; *Consultant*, Eli Lilly; *Consultant*, Sermonix; *Consultant*, Revolutions Medicine; Pedram Razavi: *Consultant*, Novartis; *Grant or Research Support*, Grail Inc; Jorge Reis-Filho: None; Fresia Pareja: None

Background: *KIT* maps to 4q12 and encodes for the receptor tyrosine kinase (RTK) KIT (CD117). *KIT,* when affected by activating somatic mutations, may result in a constitutively active RTK. *KIT* activating mutations constitute key therapeutic target in gastrointestinal stromal tumors (GISTs), melanoma and thymic tumors. We sought to investigate the presence of *KIT* activating somatic mutations in breast cancer (BCs) and to describe the clinicopathologic features of these tumors.

Design: We conducted the retrospective query of 5,575 BCs previously subjected to clinical MSK-IMPACT targeted sequencing to identify BCs harboring oncogenic/likely oncogenic somatic mutations in *KIT*. The histopathologic features of BCs harboring *KIT* oncogenic/likely oncogenic mutations were centrally reviewed. Estrogen receptor (ER)/HER2 status were retrieved from the clinical records.

Results: Seven BCs (0.13%), including 2 primary BCs (p-BCs) and 5 metastatic BCs (m-BCs), were found to harbor *KIT* oncogenic/likely oncogenic somatic mutations. These included a V559G hotspot missense mutation, 2 hotspot in-frame deletions (M552_570del and V560_578del) and four likely oncogenic missense mutations (R804W, R634Q, L793S and D792E). One p-BC was an ER+/HER2- pleomorphic lobular carcinoma of histologic grade 3, the other p-BC was an ER+/HER2+ invasive ductal carcinoma of not special type (IDC-NST) of histologic grade 3. All m-BCs harboring *KIT* activating mutations were poorly differentiated and encompassed two pleomorphic lobular carcinomas (2/5; 40%), two IDC-NSTs (2/5; 40%) and one micropapillary carcinoma (1/5; 20%). Most m-BCs 4/5 (80%) were ER+/HER2- and 1/5 (20%) was ER-/HER2-. Metastases affected liver (2/5), lymph node (1/5), skin (1/5) and chest wall (1/5). No *ESR1* mutations were identified in any of the cases. In two m-BC patients, where both the primary and metastatic BC samples were subjected to MSK-IMPACT, *KIT* activating mutations were only detected in the metastatic samples.

Conclusions: BCs harboring *KIT* activating mutations are exceedingly uncommon. These BCs are phenotypically heterogeneous, but appear to be enriched for ER+ tumors. The presence of somatic *KIT* mutations restricted to metastasis in cases for which paired p-BC and m-BC were interrogated suggests that genetic alterations in *KIT* might be late events in the evolution and/or progression in BC or might represent a mechanism of resistance in ER+ relapsed cases lacking *ESR1* mutations.

281 The Diagnostic Utility of EZH2 H-Score and Ki-67 Index in Non-Invasive Mammary Apocrine Lesions

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Disclosures: Theodore Vougiouklakis: None; Brendan Belovarac: None; Andrew Lytle: None; Luis Chiriboga: None; Ugur Ozerdem: None

Background: In diagnostic breast pathology, there is no reliable immunostain that can discern benign apocrine lesions from atypical and in situ apocrine lesions. Diagnosis of non-invasive apocrine proliferations is challenging, with current diagnoses rendered based on morphology on hematoxylin and eosin staining. Interobserver variability is significant even among subspecialists. Adjunct diagnostic immunohistochemical stains, such as high molecular weight cytokeratins which are typically useful in non-apocrine atypical or in situ lesions are not beneficial in their apocrine counterparts. Here, we set out to elucidate the diagnostic utility of EZH2 and Ki-67 immunostains as tangible tools in non-invasive apocrine proliferations.

Design: Non-invasive apocrine proliferations were subjected to EZH2 (n=38) and Ki-67 (n=25) immunostaining. Apocrine breast lesions were catalogued as follows: benign apocrine hyperplasia (BAH), atypical apocrine hyperplasia (AAH), and apocrine ductal carcinoma in situ (ADCIS). EZH2 expression was analyzed by H-scoring of nuclear EZH2 immunoexpression. Ki-67 index was quantified by calculating percentage of positive nuclei.

Results: H-scores for EZH2 ranged from 7.4-50.8 (mean: 23.5) in BAH, 4.2-99.8 (mean: 47.4) in AAH, and 112.4-288 (mean: 196.4) in ADCIS. Statistical analysis utilizing the Kruskal-Wallis test showed a significant difference (p<0.0001). Dunn's multiple comparison test demonstrated a significant difference between BAH and ADCIS (p<0.0001), and AAH and ADCIS (p=0.0003), however didn't reach a level of significance when comparing BAH and AAH. The latter association reached a statistically significant difference (p=0.0309) by applying the Mann-Whitney test. Interestingly, we observed that the basally located epithelial cells showed more robust nuclear staining when compared to adluminal cells in our pilot cohort. The average Ki-67 index was 1.6%, 4.7%, 24.7% in BAH, AAH, and ADCIS, respectively (p<0001, Kruskal-Wallis test). Dunn's multiple comparison test demonstrated a significant difference between BAH and ADCIS (p<0.0001), AAH and ADCIS (p=0.0199), and BAH and AAH (p=0.0434).

Conclusions: We demonstrate incremental EZH2 expression and Ki-67 index from BAH to AAH and robust positivity in ADCIS, suggesting EZH2 upregulation and increased Ki-67 index in atypical and in situ apocrine breast lesions compared to benign apocrine lesions. These findings suggest a diagnostic utility for EZH2 and Ki-67 indices in non-invasive apocrine mammary lesions.

282 Discordance of Oncotype DX Scores in Bilateral and Unilateral Multifocal Breast Cancers

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Disclosures: Jing Wang: None; Hui Chen: None; Isabelle Bedrosian: None; Yun Wu: None; Constance Albarracin: None

Background: Oncotype DX (DX) is a 21-gene expression profiling test that generates a recurrence score (RS) which is used to determine the efficacy of chemotherapy. The DX test has become standard of care in the management of ER-positive, lymph node-negative (ER+/LN-) breast cancer (BC). However, in ER+/LN- patients with bilateral BCs and unilateral multifocal BCs, it is not well defined when testing of these different foci is necessary to inform use of chemotherapy. Our goals were: 1. to evaluate the concordance of DX results in bilateral BCs and in unilateral multifocal BCs; and 2. to characterize pathological predictors of discordant DX RS.

Design: 1321 ER+, HER2- primary invasive BC female patients with DX performed between 2011 and 2018 were reviewed. 18 bilateral BCs patients (36 tumors) and 13 multifocal BCs patients (27 tumors) with DX testing in all tumor foci were included. Hormone receptor expression positivity was defined as ≥1% nuclear staining. HER2 level was re-evaluated according to the 2018 guideline. RS were analyzed as low risk group (RS<18), intermediate (RS 18–30), and high group (RS>30). Discordance or discrepancy was present when tumor foci for a particular patient had different RS score, histology (ductal, lobular), grade (low, intermediate, high), Ki67 index or PR staining (positive, negative).

Results: All tumors were ER+ by IHC/DX and HER2- by IHC/FISH/DX. DX RS were discordant in 50% of bilateral BCs and 54% of multifocal BCs. However, bilateral BCs were present in significantly older (p<0.01) patients and have more discordant histologic type (p<0.05) compared to multifocal BCs. Other histologic parameters were not significantly different between bilateral BCs and multifocal BCs, as both groups exhibited similar histologic grade, Ki67 and PR between foci. Interestingly, concordant DX RS were more likely to be associated with similar histologic grade and Ki67 in 89% (8/9) of bilateral BCs and 100% (6/6) of multifocal BCs. In contrast, discordant DX RS were more variable having similar histology grade in only 56% (5/9) and 57% (4/7) and similar Ki67 in 44% (4/9) and 43% (3/7) of bilateral and unilateral multifocal BCs, respectively.

Figure 1 - 282

Table 1. Clinicopathologic characteristics of bilateral and unilateral multifocal breast cancers.

	Bilateral n=18	Unilateral multifocal n=13	P value
Age, Median (range)	59 (44-72) yo	49 (36-63) yo	<0.01
Tumor Size, Median (range)	17 (3-90) cm	12 (4-46) cm	0.41
Histology			<0.05
Similar	10 (56%)	12 (92%)	
IDC-IDC	9	10	
IDC-ILC	1	2	
Discrepant	8 (44%)	1 (8%)	
Nottingham Histologic Grade			1.00
Similar	13 (72%)	10 (77%)	
Discrepant	5 (28%)	3 (23%)	
Ki67			1.00
Similar	12 (67%)	9 (69%)	
Discrepant	6 (33%)	4 (31%)	
PR			0.67
Similar (PR+/PR+)	13 (72%)	11 (85%)	
Discrepant (PR+/PR-)	5 (28%)	2 (15%)	
Oncotype DX RS			1.00
Similar	9 (50%)	6 (46%)	
Discrepant	9 (50%)	7 (54%)	

Conclusions: Discordance of DX recurrence scores is common in both bilateral BC and unilateral multifocal BC. The cases with discordant DX RS are more likely to have variable and discrepant histologic grade and Ki67. Therefore, testing multiple foci should be considered particularly when histopathologic features are discrepant.

283 Combined Therapy with Cytokine-Induced Killer Cells and Oncolytic Adenovirus Expressing Anti-p21Ras ScFv Induce Enhanced Antitumor Activity in Breast Cancer

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Disclosures: Peng Wang: None; Xinyan Pan: None; Qiang Feng: None; Julun Yang: None

Background: Breast cancer is one of the essential diseases that pose a severe threat to women's. *RAS* mutations and overexpression of the p21Ras protein is the leading causes of cancer development and progression, which has made the *RAS* gene and p21Ras as essential targets for therapy of *RAS*-driven cancers. Previously, we constructed KGHV300, which carries the anti-p21Ras single-chain variable fragment (scFv) and two tumor-specific promoters, the human telomerase reverse transcriptase (hTERT) and hypoxia response element (HRE) promoters, and it exhibited significant antitumor activity in several tumors including breast cancer. However, it has low targeting and toxic side effects on healthy tissues. It has been reported that cytokine-induced killer (CIK) cells can be used as a cell carrier to carry poxviruses, successfully evading the body's immune surveillance and passing the vascular endothelium and tumor tissue barriers. Thus, the use of CIK as a carrier for adenovirus maybe solve the shortcomings of the KGHV300.

Design: Firstly, to make the recombinant adenovirus infect CIK cells, we replaced the Fiber5 gene of skeleton virus plasmid pBHGE3 with Fiber35 gene of the plasmid pUC-F35 and formed a new skeleton virus plasmid pBHGE3-F35. Secondly, The plasmids pBHGE3-F35 and the shuttle virus plasmid pXC2p-scFv (Previously constructed) were co-transfected into HEK293 cells. The recombinant adenovirus was purified by Cesium chloride density gradient centrifugation and was called KGHV500. Thirdly, in vitro, the human breast cancer cell line MDA-MB-231 was employed to investigate the anti-tumor activity of KGHV500 using MTT, wound healing, and transwell invasion assays. Finally, in vivo, MDA-MB-231-transplanted tumors in nude mice were constructed and utilized to evaluate the treatment effect of the combination of CIK cells with KGHV500.

Results: KGHV500 was successfully constructed and purified. In vitro, KGHV500 could significantly inhibit proliferation, migration, and invasiveness and promote cell apoptosis in MDA-MB-231 cells. In vivo, CIK combined with KGHV500 not only enhanced the anti-tumor effect on MDA-MB-231 cell transplanted tumor but also reduced the toxic side effects of KGHV500 on various organs.

Figure 1 - 283

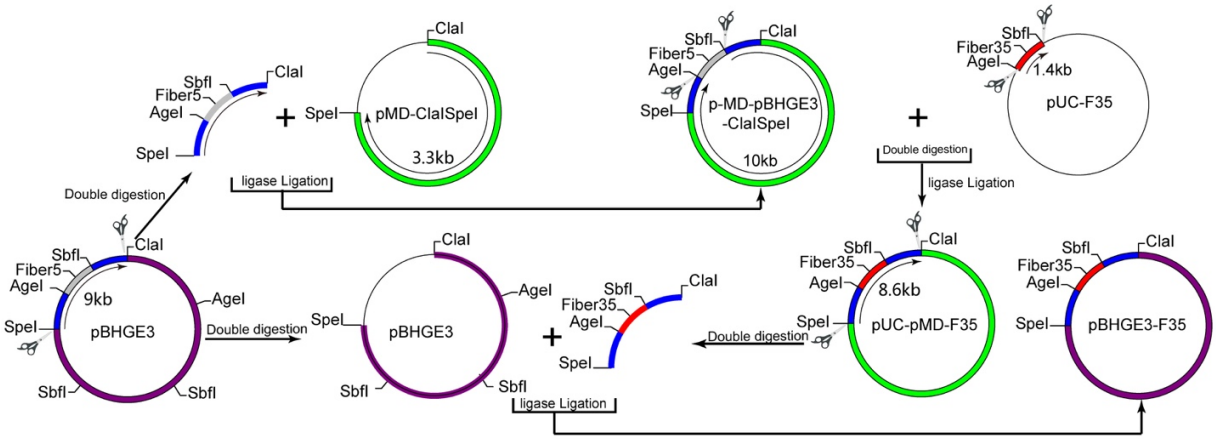
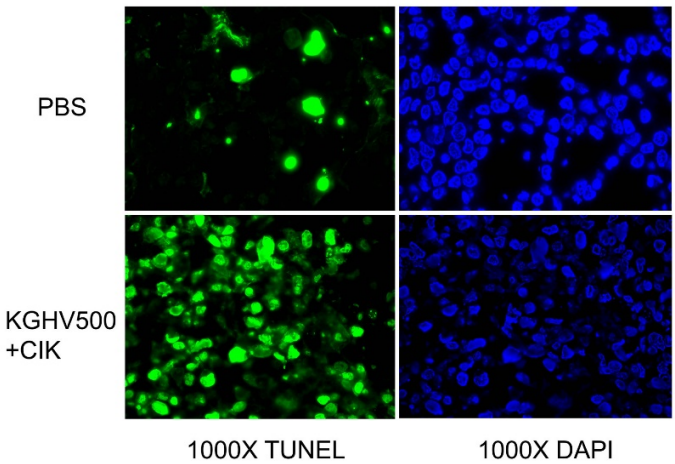


Figure 2 - 283



Conclusions: CIK combined with KGHV500 could enhance the anti-breast cancer effect and safety, and it can be used as a strategy for the treatment of *RAS*-driven breast cancer in the future.

284 Impact of Progesterone Receptor (PR) and Androgen Receptor (AR) on Aromatase Inhibitor Treated Estrogen Receptor (ER) Positive Metastatic Breast Carcinomas

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Disclosures: Xi Wang: None; Sindhuja Kadambi: None; Laura Eckert-Davis: None; Myla Strawderman: None; Philip Meacham: None; Hasan Khatib: None; Qi Yang: None; Ajay Dhakal: None

Background: Breast cancer (BC) is a hormone related cancer, among which 80% are ER+, 70% PR+ and up to 90% AR+. Aromatase inhibitor (AI), which inhibits conversion of androgens to estrogen at tissue level, has been shown to prolong survival of women with early stage or metastatic ER+ BC. PR and AR expressions seem to provide good prognosis to ER+ BC, but predictive value of AR and PR in ER+ metastatic BC in the setting of aromatase inhibition are not well established.

Design: Imunohistochemical stain for AR was performed on the archival metastatic breast cancer (MBC) samples. Only women with biopsy proven ER+HER2- MBC, who have received AI +/- CDK4/6 inhibitors in the first line were included. AR staining result was recorded as negative (<1% of tumor cells stained) orpositive (=/> 1% tumor cells stained) according to the standard for evaluation of ER. PR status was recorded from the original pathology report. Chart review was performed to obtain clinical data. Progress-free survival (PFS) was defined as the time (months) between first AI and either progression or death, or right censored at last follow-up. Unadjusted PFS was estimated by the Kaplan-Meier method. Median PFS is reported with 95% confidence intervals (CI). The log-rank test compared PFS over the follow up period.

Results: Eighty-five cases met the eligibility criteria and had sufficient clinical follow up information. Thirty-eight (44.7%) had received chemotherapy and 50 (58.8%) had received anti-estrogen therapy in the perioperative settings of BC. Thirty-six (42.4%) patients received a CDK4/6 inhibitor combined with AI. Valid AR staining was obtained on 68 cases (56AR+, 12 AR- cases) and valid PR information was available on 79 cases (46 PR+, 33 PR- cases). Median PFS on AIs as the first line therapy was 14.8 (10.7, 24.5) months in AR+ BC vs. 13.4 (4.9, Not evaluable) months in AR- BC (p=0.68) and was 18.8 (14.8, Not evaluable) months in PR+ BC vs. 12.9 (9.0, 20.6) months in PR- BC (p=0.0145). Among sixty-four cases with both valid AR and PR status, there was no evidence of an association of these two receptors (p=0.21).

Conclusions: There was no significant difference between the clinical outcomes of AR+ ER+ MBC as compared to AR- ER+ MBC on AI as first line treatment. However, PR+ ER+ MBC was associated with better PFS as compared to PR- ER+ MBC on first line AI treatment suggesting a predictive value of PR with aromatase inhibition. A larger study is needed to confirm these findings.

285 Molecular Characteristics of Breast Carcinoma with Neuroendocrine Differentiation Based on Whole Exome Sequencing

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Disclosures: Yani Wei: None; Menglan Zhang: None; Xue-Xuan Ke: None; Qiuyang Jing: None; Jiaxiu Yu: None; Bing Wei: None

Background: Invasive breast carcinoma with neuroendocrine differentiation (BCND) is defined as the primary mammary neoplasm with expression of chromogranin (CgA) proteins and/or synaptophysin (Syn) to a greater or a lesser degree, accounting for approximately 2% to 5% of breast cancer. Compared with invasive breast carcinomas of no special type (NST), it is more aggressive and has a worse prognosis. At present, molecular genetic research on BCND is limited and the molecular mechanism of BCND development needs to be further clarified.

Design: We reviewed 652 invasive breast cancers with fresh tissue samples from 2013-2018 and 38 cases with BCND diagnosed in 2018. Then, we collected cases with ≥50% tumor cells expressing CgA and/or Syn. Finally, the whole-exome sequencing (WES) was performed on 14 pairs (28 fresh samples) and 3 paraffin samples of BCND to identify key mutations in this unusual neoplasm.

Results: The clean reads of 28 fresh and 3 paraffin samples accounted for more than 90% of the original testing data, the average sequencing error rate was less than 0.1%, and the quality of the sequencing data was more than Q30 (≥80%). Genetic analysis related to the characteristics of BCND shows that the most frequent mutation type (14/14) is C>T/G>A and the high frequency mutant genes are *GATA3* (14.3%), *UFL1* (14.3%), *KRT32* (14.3%). High-frequency CNV analysis shows that BCND has amplification of 11q, 8q, 17q, 19q and deletion of 17q and 11q. In addition, genetic analysis related to tumorigenesis shows that BCND has the following specific genes: (1) tumor susceptibility genes: *PIK3CB, CARS, TSHR, EPHB2, GAS7, TNPO1*; (2) tumor-driven genes: *PLEC, CHD3, TP53, DNMT1, TRRAP, TRIO, STAT3*; (3) heterozygous deletion gene: *ZIC1, KDM3B, UFL1, NKX3-1*. The mutated genes related to drug resistance in the BCND samples are: *PIK3CA, ABCC6, CAT, AKT1, TP53*. The sequencing results in BCND show that there are high frequency mutant genes with targeted drugs in other types of tumors: *G6PD, CACNA1G, PDE3A, G6PD, ACE, CBFB, PIK3CA.*

Table 1. Summary of available clinicopathologic features in BCND

Case no.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sex /Age (y)	F 47	F 49	F 75	F 52	F 60	F 35	F 49	F 32	F 53	F 41	F 52	F 59	F 54	F 46
Laterality	L	R	R	R	R	L	L	R	L	L	L	R	R	R
TNM stage	I	IIIA	IIA	IIA	IIA	IIB	IV	IIIC	IIIC	IIB	IIA	IIA	IIA	IIC
Grading	2	3	3	3	2	3	3	3	3	3	3	3	2	3
ER	+	-	+	+	+	+	+	+	+	+	+	+	+	+
PR	+	-	+	+	+	+	+	+	+	+	+	+	+	+
HER2 IHC	2+	3+	0	1+	2+	2+	2+	1+	3+	3+	0	3+	2+	1+
HER2 FISH	ND	ND	ND	ND	N	N	N	N	A	ND	N	ND	N	ND
Ki67 (%)	30	35	30	30	35	80	50	3	45	50	75	75	20	30
Menstrual status	Y	Y	N	N	N	Y	Y	Y	N	Y	Y	N	Y	Y
Surgical procedure	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Chemotherapy	Y	Y	N	Y	N	Y	Y	Y	Y	Y	Y	Y	N	Y
Radiotherapy	N	Y	N	N	N	Y	Y	Y	Y	Y	N	N	N	Y
Endocrine therapy	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y
HER2-targeted therapy	N	N	N	N	N	N	N	N	N	N	N	Y	N	N
Follow-up (months)	D 17	D 62	D 37	D 35	D 29	D 20	LB 9	D 46	D 46	D 60	D 63	D 7	D 7	D 10

L, left; R, right; F, female; Y, Yes; N, No; M, Mastectomy; A, amplification; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; ER, estrogen receptor; PR, progestational hormone; HER2, c-erbB2; D, no evidence of disease; LB, bone and lymph node metastasis; ND, not done.

Figure 1 - 285

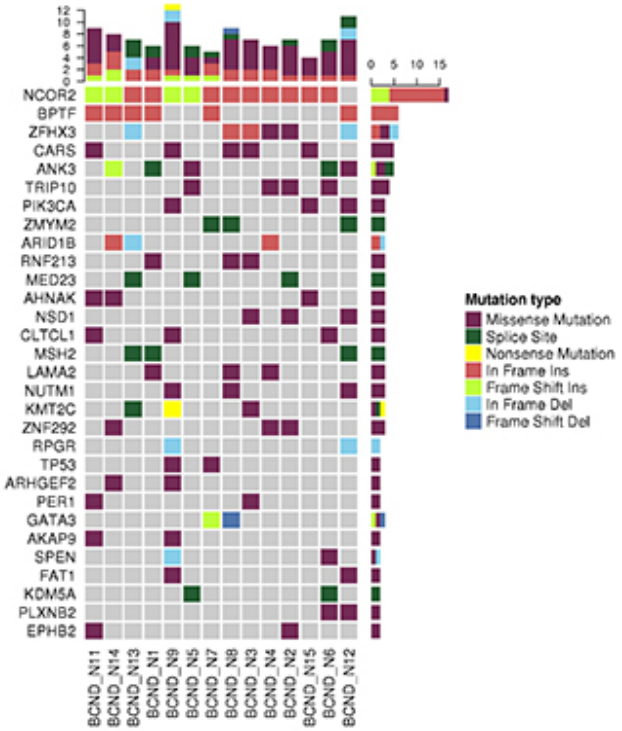
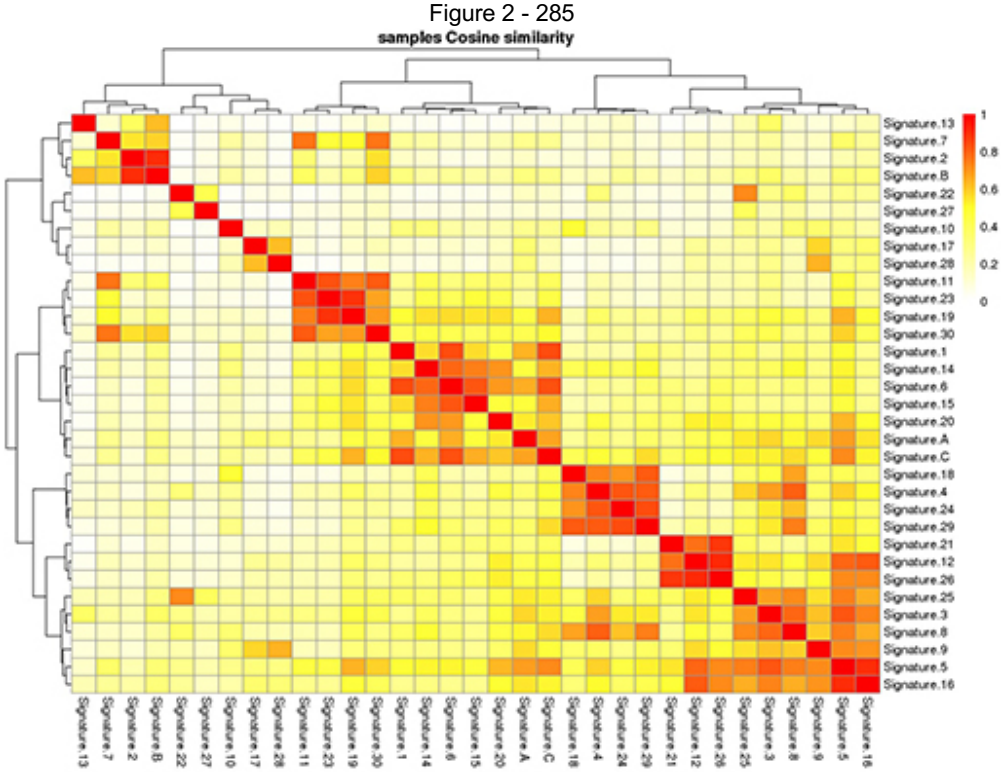


Figure 2 - 285



Conclusions: According to literature review, the current molecular studies of BCND are few and the number of cases included in each study is small. In this study, we showed a relatively large number of WES results of BCND. The WES results indicate that BCND has more specific gene changes, supporting BCND as an independent invasive breast cancer subtype. Higher throughput, massively parallel sequencing on larger cohorts are further needed to investigate the molecular characteristics of BCND.

286 Poor Response to Neoadjuvant Chemotherapy in Metaplastic Breast Carcinoma

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Disclosures: Willard Wong: None; Edi Brogi: None; George Plitas: *Advisory Board Member*, Tizona Therapeutics; *Advisory Board Member*, Merck; Mark Robson: None; Larry Norton: None; Monica Morrow: *Speaker*, Genomic Health; Hannah Wen: None

Background: Metaplastic breast carcinoma (MBC) is a rare subtype of breast cancer characterized by the presence of squamous, spindle or mesenchymal differentiation. Most MBCs are triple negative and patients with MBCs frequently undergo neoadjuvant chemotherapy (NAC). However, response to NAC has not been well studied and reported response rates have been variable. The aim of this study was to evaluate response to NAC in a retrospective series of MBC.

Design: MBCs treated with NAC at our center from 2003-2018 were retrospectively reviewed. The association between clinico-pathologic features and response to NAC was analyzed by Fisher's Exact test. Survival outcomes were estimated by Kaplan-Meier method.

Results: 38 patients with MBC treated with NAC were identified. Of cases with reported subtype, 17 (45%) were matrix-producing, 8 (21%) squamous cell, 6 (16%) spindle cell, and 5 (13%) with mixed metaplastic components. No low-grade variants of MBC were included. Mean age was 50 years, 84% were clinical stage II-III, and 42% were clinically node positive. 76% (29/38) were triple negative or with low (<10%) hormonal receptor expression and HER2 negative on pre-NAC biopsy. 76% patients received a doxorubicin, cyclophosphamide and taxol NAC regimen. Of 33 cases with documented responses, 49% showed clinico-radiological progression or no clinical response on NAC, and 51% showed partial response. Two patients were downstaged from initially inoperable to lumpectomy. 74% of patients underwent mastectomy post NAC.

No patients achieved pathologic complete response (pCR). One patient (matrix-producing type) had breast pCR but had residual nodal disease. Among patients with biopsy-proven node positive disease pre-NAC, 19% (3/16) had nodal pCR. High nuclear grade ($p = 0.021$) and matrix-producing subtype ($p = 0.032$) were associated with clinico-radiological response. Residual cancer burden (RCB) was assessed in 20 cases, 5% had RCB I, 80% had RCB II, and 15% had RCB III. Median follow-up was 19 months. 11 patients had recurrence (2 local, 10 distant). RCB was the only factor significantly associated with recurrence-free, distant recurrence-free, overall and disease-specific survival.

Table 1. Clinicopathological features associated with clinical and radiological response to NAC

		Clinical and Radiological				
	(Available Data)	**Progression (%)**	**No Response (%)**	**Improvement (%)**	**Unknown Response (%)**	**p value**
Age (years)	≤50 (23)	3 (8)	5 (13)	11 (30)	4 (11)	0.3029
	>50 (15)	6 (16)	2 (5)	6 (16)	1 (3)	
Size	cT1 (5)	0	0	2 (5)	3 (8)	0.9631
	cT2 (23)	6 (16)	5 (13)	10 (26)	2 (5)	
	cT3/4 (9)	2 (5)	2 (5)	5 (13)	0	
	Unknown (1)	1 (3)	0	0	0	
Nuclear Grade	1 (0)	0	0	0	0	**0.0213**
	2 (6)	0	0	6 (16)	0	
	3 (23)	5 (13)	7 (18)	7 (18)	4 (11)	
	Unknown (9)	4 (11)	0	4 (11)	1 (3)	
Tumor Classification	Pure metaplastic (15)	6 (16)	2 (5)	6 (16)	1 (3)	0.3029
	Mixed metaplastic and NST (23)	3 (8)	5 (13)	11 (30)	4 (11)	
Metaplastic Subtype	Matrix (17)	2 (5)	2 (5)	11 (30)	2 (5)	**0.0321**
	Spindle (6)	3 (8)	0	2 (5)	1 (3)	
	Squamous (8)	3 (8)	2 (5)	2 (5)	1 (3)	
	Mixed (5)	0	3 (8)	1 (3)	1 (3)	
	Unknown (2)	0	0	2 (5)	0	
Residual Cell Burden	I (1)	0	0	0	1 (3)	0.5588
	II (16)	3 (8)	3 (8)	9 (24)	1 (3)	
	III (3)	1 (3)	1 (3)	1 (3)	0	
	Unknown (18)	5 (13)	3 (8)	7 (18)	3 (8)	
Residual Tumor Cellularity	≤50 (11)	1 (3)	1 (3)	8 (21)	1 (3)	0.1498
	>50 (11)	4 (11)	3 (8)	3 (8)	1 (3)	
	Unknown (16)	4 (11)	3 (8)	6 (16)	3 (8)	
Tumor Bed Area	≤900 mm^2 (12)	0	2 (5)	8 (21)	2 (5)	0.1697
mean: 1491, median: 912	>900 mm^2 (12)	4 (11)	2 (5)	5 (13)	1 (3)	
	Unknown (14)	5 (13)	3 (8)	4 (11)	2 (5)	
Pre-NAT Biomarker Status	HR >10%/HER2- (7)	1 (3)	2 (5)	3 (8)	1 (3)	0.9673
	HR 1-10% /HER2- (4)	1 (3)	0	2 (5)	1 (3)	
	HR 1-10%/HER2+ (1)	0	0	1 (3)	0	
	Triple Negative (25)	7 (18)	5 (13)	10 (26)	3 (8)	
	HR-/HER2+ (1)	0	0	1 (3)	0	
Post-NAT Biomarker Status	HR+ (4)	0	1 (3)	3 (8)	0	0.6329
	Triple Negative (28)	8 (21)	5 (13)	11 (30)	4 (11)	
	HER2+ (1)	0	0	1 (3)	0	
	Unknown (5)	1 (3)	1 (3)	2 (5)	1 (3)	

Conclusions: Patients with metaplastic carcinoma had poor clinical and pathological response to NAC with some patients with progression while on NAC. Most patients ultimately underwent mastectomy. Metaplastic histology should be taken in consideration in the initial treatment planning and RCB was an important prognostic factor.

287 A Feasibility Study in The Automated Quantification of HER2 Gene Amplification in Breast Cancer Using Chromogenic In Situ Hybridization Whole Slide Images

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Disclosures: Willard Wong: None; Hossain Shakhawat: None; Matthew Hanna: None; Takashi Inoue: None; Benjamin Stueben: None; Kareem Ibrahim: None; Alexei Teplov: None; Marcia Edelweiss: None; Edi Brogi: None; Meera Hameed: None; Dara Ross: None; Yukako Yagi: None

Background: HER2 gene amplification is seen in up to 20% of breast cancers (BC). HER2 is a predictive and prognostic biomarker in BC and accurate assessment of the HER2 status is essential. Advantages of CISH compared to FISH include use of a light microscope, appreciation of morphology and lower cost; however, manual evaluation is labor intensive and time consuming. We aim to study the practicality of automated quantification of HER2 CISH on whole slide images (WSI).

Design: Thirty-five cases of invasive or metastatic BC with prior IHC and/or FISH testing were randomly selected to include Groups 1 and 5 FISH cases categorized by the 2018 ASCO/CAP guidelines. Subsequent manual assessment by dual-probe CISH was performed and categorized as Groups 1 to 5. CISH slides were scanned at 40x (0.13 um/pixel) by P250 and P1000 3DHistech (Hungary). Regions of interest (ROI) containing invasive cells were manually annotated and analyzed with SHIMARIS PACQ V1.2 (in-house application) for automated enumeration of CISH probes and HER2 status classification. The manual CISH results were compared to the automated CISH results.

Results: Automated evaluation was performed on 35 cases, including 26 (74%) excisions/mastectomies, and 9 (25%) biopsies. 28 cases were primary diagnoses, and 7 were metastases/recurrences. The mean age was 55 years old (range 30-83). Histologic subtype was: 28 (80%) ductal, 6 (17%) mammary, and 1 (3%) classic lobular. Of 27 cases with a documented grade, 15 were poorly, 1 moderate-poorly, 10 moderately, and 1 was well differentiated.

Automated CISH was concordant with manual CISH in 33/35 (94%) cases (Table 1). One case had a HER2/CEP17 ratio of 2.00 and HER2 copy number (CN) per nuclei of 4.92 by manual, representing a borderline case for Group 1 or 4, and 1.72 ratio and 4.21 HER2 CN (Group 4) by automated CISH. Another case showed a 3.46 ratio and 7.95 HER2 CN by manual (Group 1), and 3.19 ratio and 3.87 HER2 CN by automated CISH (Group 2). This case showed overlapping of nuclei, occasional tissue section folding, and weak CEP17 signal.

Table 1: Manual CISH vs Automated CISH

		Automated CISH				
		Group 1	Group 2	Group 3	Group 4	Group 5
Manual CISH	Group 1	22	1	0	1	0
	Group 2	0	0	0	0	0
	Group 3	0	0	0	0	0
	Group 4	0	0	0	0	0
	Group 5	0	0	0	0	11

Conclusions: We have demonstrated the feasibility of automated HER2 CISH evaluation. Automated HER2 CISH has excellent concordance with manual CISH. Careful assessment of ROI, tissue processing, and signal intensity is necessary and emphasizes the importance of morphologic correlation in CISH. Further study in the automated platform could provide clinical efficiencies and may enable automated analysis in other tumors (i.e. gastric) using HER2 prognostication.

288 Abstract Withdrawn

289 Impact of the Updated 2018 ASCO/CAP HER2 Guidelines: A Retrospective Study

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Background: The updated 2018 ASCO/CAP (the American Society of Clinical Oncology/College of American Pathologists) clinical practice guidelines on HER2 testing in breast cancer recommend using more rigorous criteria by integrating immunohistochemical (IHC) and fluorescence in situ hybridization (FISH) results and recounting FISH, aiming to eliminate the “equivocal” category. We set out to examine the impact of these guidelines on HER2 FISH testing at our institution.

Design: HER2 FISH tests requiring a second count from 1/5/2015 to 31/8/2019 were identified. Tests performed after 1/7/2018 followed the 2018 guidelines, while tests performed prior to this date followed the 2013 guidelines as well as in- house algorithm recommending a second read if the HER2/CEP17 ratio is near a decision threshold or if the mean HER2 signal is ≥5 and <6. Additionally, equivocal cases based on a single read were also reclassified.

Results: A total of 429 cases were identified and divided into 3 cohorts: 1) 76 cases after the introduction of the 2018 guidelines, requiring a second observer to recount at least 20 cells. The second read altered the FISH group designation in 14 cases (18%). However, when the results of the first and second counts were combined, the final group designation only changed in 2 cases (3%). Most cases (73/76, 96%) were considered negative; 2) 111 cases that followed the 2013 guidelines and were read by two observers according to in-house policy, including 106/111 (95%) equivocal, 3/111 (3%) positive and 2/111 (2%) negative cases. The 2018 guidelines reclassified all 106 equivocal cases as negative. The 2 negative and 3 positive cases per the 2013 guidelines remained unchanged under the 2018 guidelines; 3) 242 cases that were classified as equivocal based on the 2013 guidelines with 2+ IHC and FISH scores by a single observer. The 2018 guidelines reclassified 1 case as positive and 5 cases as negative. The positive case had a biphasic pattern with both amplified and non-amplified areas. Overall, a significant number of the cases (236, 98%) required a FISH recount to establish the final HER2 status.

Table 1. Classification of HER2 FISH results by 2013 and 2018 ASCO/CAP guidelines. Group 1 (amplified): HER2/CEP17 ratio ≥2.0; mean HER2 ≥4.0. Group 2 (monosomy): HER2/CEP17 ratio ≥2.0; mean HER2 <4.0. Group 3 (polysomy): HER2/CEP17 ratio <2.0; mean HER2 ≥6.0. Group 4 (borderline): HER2/CEP17 ratio <2.0; mean HER2 ≥4.0 and <6.0. Group 5 (non-amplified): HER2/CEP17 ratio <2.0; mean HER2 <4.0.

					HER2 status by ASCO/CAP 2018		HER2 status by ASCO/CAP 2013		
	HER2 FISH groups	Reader 1	Reader 2	Combined	Positive	Negative	Positive	Negative	Equivocal
Cohort 1	Group 1	1 (1.3%)	4 (5.3%)	1 (1.3%)	1	0			
	Group 2	0 (0%)	0 (0%)	0 (0%)	0	0			
	Group 3	3 (4%)	3 (3.9%)	2 (2.7%)	2	0			
	Group 4	72 (94.7%)	65 (85.5%)	72 (94.7%)	0	72			
	Group 5	0 (0%)	4 (5.3%)	1 (1.3%)	0	1			
	Total	76 (100%)	76 (100%)	76 (100%)	3 (3.9%)	73 (96.1%)			
Cohort 2	Group 1	3 (2.7%)	6 (5.4%)	2 (1.8%)	2	0	2	0	0
	Group 2	1 (0.9%)	0 (0%)	0 (0%)	0	0	0	0	0
	Group 3	0 (0%)	5 (4.5%)	1 (0.9%)	1	0	1	0	0
	Group 4	107 (96.4%)	99 (89.2%)	107 (96.4%)	0	107	0	1	106

	Group 5	0 (0%)	1 (0.9%)	1 (0.9%)	0	1	0	1	0
	Total	111 (100%)	111 (100%)	111 (100%)	3 (2.7%)	108 (97.3%)	3 (2.7%)	2 (1.8%)	106 (95.5%)
Cohort 3	Group 1	1 (0.4%)			1	0	0	0	1
	Group 2	1 (0.4%)	---> for 2nd read		0	0	0	0	1
	Group 3	0 (0%)			0	0	0	0	0
	Group 4	235 (97.1%)	---> for 2nd read		0	0	0	0	235
	Group 5	5 (2.1%)			0	5	0	0	5
	Total	242 (100%)			1 (0.4%)	5 (2.1%)	0 (0%)	0 (0%)	242 (100%)

Conclusions: Our study suggests that the implementation of the 2018 ASCO/CAP guidelines causes a significant increase in the number of HER2-negative cases that were previously designated as equivocal. With the altered testing algorithms, the equivocal category is effectively eliminated and the assessment of HER2 status is simplified, especially in cases with non-classical amplification patterns.

290 HER2 FISH Signal Degradation: A Ten Year Retrospective Study

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Disclosures: Jessie Wu: None; Gulisa Turashvili: None; Gulisa Turashvili: None; Gulisa Turashvili: None; Gulisa Turashvili: None

Background: Approximately 25% of invasive breast carcinomas are positive for human epidermal growth factor receptor 2 (HER2). Fluorescence in situ hybridization (FISH) analysis is recommended for equivocal cases via immunohistochemistry. However, there is a dearth of literature on the retention and storage requirements for HER2 FISH slides. Existing guidelines vary widely among countries and laboratories with no supportive evidence. This study aims to determine the degradation of HER2 FISH signals in an effort to determine optimal retention time and storage conditions.

Design: Dual-probe HER2 FISH slides from March 2009 to June 2019 were retrieved to assess the presence, intensity and quantity of the signals under dual and single Chroma Filters (PathVysion HER2 DNA Probe Kit; CEP17 probe - Vysis CEP17 SpectrumGreen Vysis, HER2 probe - LSI HER2/neu Spectrum Orange). At our institution, FISH slides are placed in slideboxes and stored in -80C freezers for at least 4 years. Older slides are stored at room temperature.

Results: After excluding HER2 FISH slides that were deemed uninterpretable due to technical issues, a total of 6255 slides were assessed. Slides from 2009 to 2014 were stored at room temperature, while slides from 2015 to 2019 were stored in -80C freezers. Slides stored in freezers showed retention of both the green and the orange signals. Slide stored at room temperature demonstrated significant decrease in the signal retention rate and the loss of signal did not progress in a linear fashion. The green CEP17 signal was quenched much faster than the orange HER2 signal.

Table 1. HER2 FISH signal degradation by year							
Year	Total Number of cases	Number of Cases with retained orange and green signal	Percentage (%)	Number of cases with no signal	Percentage (%)	Number of cases with loss of green signal only	Percentage (%)
2019	186	186	1.0	0	0.0	0	0.0
2018	367	364	99.2	1	0.3	0	0.0
2017	430	420	97.7	5	1.2	2	0.5
2016	613	599	97.7	3	0.5	0	0.0
2015	647	640	98.9	0	0.0	1	0.2

2014	355	84	23.7	51	14.4	58	16.3
2013	340	144	42.4	34	10.0	26	7.6
2012	572	225	39.3	9	1.6	176	30.8
2011	1116	77	6.9	49	4.4	579	51.9
2010	1142	489	42.8	55	4.8	265	23.2
2009	487	8	1.6	103	21.1	202	41.5

Conclusions: Our study is the first to demonstrate HER2 FISH signal degradation with time and slide storage conditions. Storing HER2 FISH slides in -80C freezer allows for retention of both HER2 and CEP17 signals. At room temperature, the signals start to degrade with CEP17 signals lost at a faster rate. The results of the study may be used in official guidelines for retention time and storage conditions for HER2 FISH slides.

291 Neuroendocrine Neoplasms (NENs) of the Breast Have Poorer Prognosis than Invasive Carcinoma of No Special Type (NST)

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Disclosures: Libo Yang: None; Madhuchhanda Roy: None; Constance Albarracin: None; Lei Huo: None; Isabelle Bedrosian: None; Hong Bu: None; Yun Wu: None

Background: A uniform classification framework for neuroendocrine neoplasms (NENs) in all the organ systems has been recently proposed by an International Agency for Research on Cancer (IARC) and World Health Organization (WHO) expert panel. This classification framework will also be applied to NENs of the breast in the upcoming new WHO classification of the tumors of the breast. Based on the new classification system, the NENs of the breast will be divided into well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinoma (NEC), which would include large cell NEC and small cell carcinoma. NETs will be furthered graded as G1, G2 or G3 based on the mitotic count and/or Ki-67 proliferation index.

Design: The surveillance, epidemiology and end results (SEER) database released on November 2018 was used for this study. Between 2003 and 2016, 296 NENs (NET=180, NEC=116) of the breast and 462,055 of invasive carcinoma, NST were identified. Survival analysis was performed for disease specific survival (DSS) and overall survival (OS). Survival for NET, NEC and NST were compared with different histology, grade and clinical stage.

Results: NET and NEC of the breast had significantly worse disease-specific survival (DSS) and overall survival (OS) than invasive carcinoma, NST (p<0.001) (Figures 1A and 1B). Within the NETs of the breast, the grade I, II, III NETs had worse DSS and OS than corresponding grade I, II, III invasive carcinoma, NST (all p<0.001) (Figures 1A and 1B). Within the same clinical stage, stage I, II, III NETs and NECs of the breast had worse DSS and OS than corresponding stage I, II, III invasive carcinoma, NST (p<0.001) (Figures 2A -2F).

Figure 1 - 291

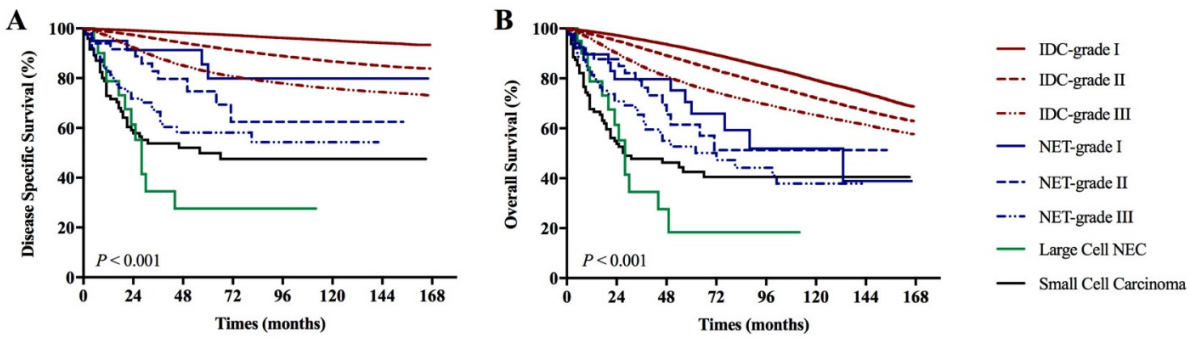
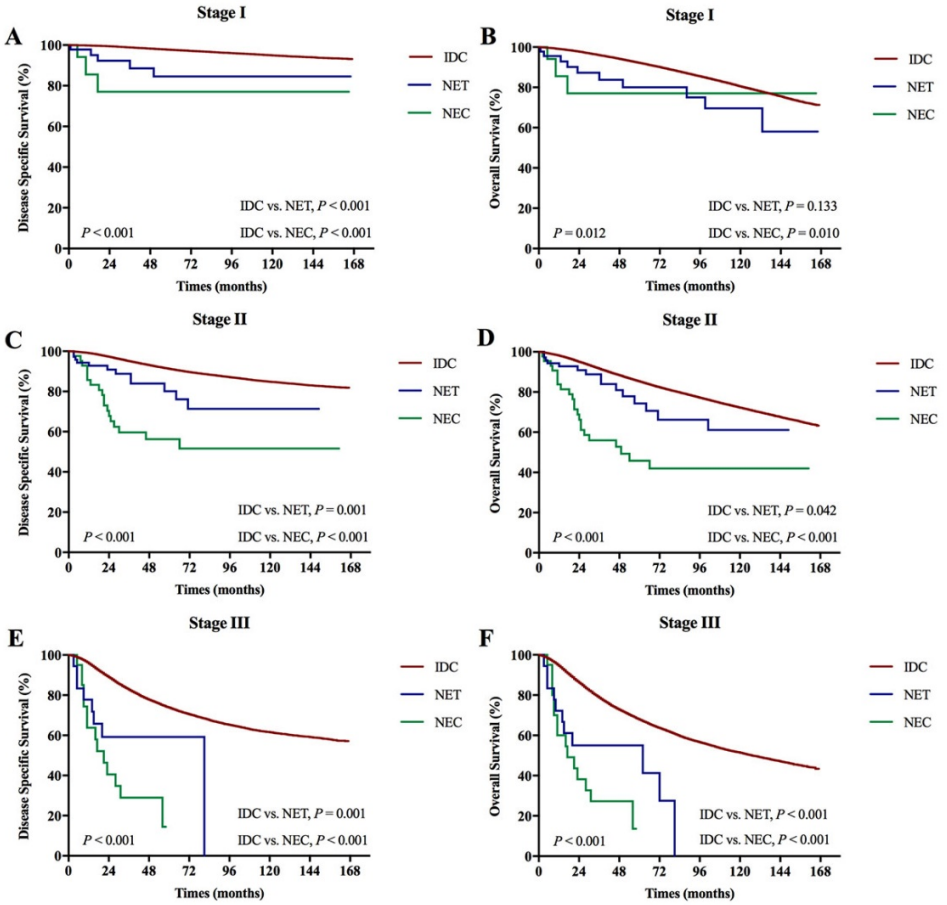


Figure 2 - 291



Conclusions: The universal classification framework for NEN allowed us to further refine the breast carcinoma with neuroendocrine differentiation as a unique pathologic and clinical entity, which has worse clinical outcome compared to invasive carcinoma, NST.

292 Active Surveillance Clinical Trial for Mammary Low Risk DCIS: The Implication of the Degree of Central Necrosis

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Disclosures: Haiying Zhan: None; Marie Quinn: None; Xuan Peng: None; Li Yan: None; Thaer Khoury: None

Background: In the current active surveillance clinical trial for low risk DCIS, specifically COMET trial, comedo necrosis was recently omitted as an exclusion criterion. The purpose of this study is to investigate the consequences of this change by correlating the degree of DCIS central necrosis (CN) and the rate of upgrade on the excisional biopsy (EB).

Design: We applied the clinical inclusion criteria of the COMET clinical trial on our patients who were mammographically screened between 2007 and 2017. Histologic findings on the core needle biopsy (CNB) were recorded. For the degree of CN, we measured the diameter and the area size for both the CN and the ductal space. Then we calculated the ratio diameter of the CN/the ductal space, and the ratio area size of CN/ ductal space. The span of calcifications was measured on the mammography images. Upgrade to high risk residual disease was identified when invasive carcinoma (IC) and/or high grade DCIS was identified on the EB. We defined comedo necrosis using 30% cutoff. We correlated the recorded variables with the upgrade, first including all cases (n=129), and then excluding cases with comedo necrosis (n=76). We also correlated the pathologic and radiologic variables with the IC and with no residual disease separately.

Results: In 129 DCIS cases, 26 (20.2%) had upgrade, 12 (9.3%) to IC, and 14 (10.9%) to high grade DCIS. The rate of upgrade including cases with no comedo necrosis was 13 of 76 (17.1%). When all cases were included in the analysis, larger calcification span correlated with upgrade with median and range of 1.7 (0.1 to 3.3) for upgrade vs. 0.9 (0.1 to 5.6) for no upgrade (p=0.02). The best cutoff of

calcification span on mammography to predict upgrade was 1.1-cm with area under the curve (AUC) of 0.648. However, when we excluded cases with comedo necrosis, none of the variables was statistically significant. The following variables predicted IC including number of spaces with CN (p=0.047) and diameter ratio of CN (p=0.041) but not the area size ratio (p=0.074). The following variables predicted no residual disease including less number of spaces with CN (p=0.006), smaller CN diameter ratio (p=0.004), and smaller calcification span (p<0.001).

Conclusions: Changing the inclusion criteria in the COMET trial to include any degree of necrosis did not affect on the rate of upgrade. However, the criteria of the span of calcifications may need to be reviewed. We recommend conducting a larger multi-institutional study to evaluate our results.

293 Touching Tumor-Infiltrating Lymphocytes in Low Risk Ductal Carcinoma In Situ Detected in a Setting Resembling Active Surveillance Clinical Trials for Low Risk DCIS Correlates with Upgrade to High Grade DCIS

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Background: Active surveillance clinical trials for low risk DCIS have been recently initiated. It is important to identify ways to predict cases with high risk residual disease. Dense touching tumor infiltrating lymphocytes (TILs) has been shown to correlate with the nuclear grade. We hypothesize that higher number of touching TILs in a low risk DCIS detected in a core needle biopsy (CNB) could predict a high grade (HG) DCIS in the excisional biopsy (EB).

Design: We applied the clinical inclusion criteria of the COMET clinical trial on our patients who were mammographically screened between 2007 and 2017. Histologic findings on the CNB were recorded. Touching TILs were assessed by counting the number of TILs touching the ductal basement membrane or away from it by one lymphocyte cell thickness. TILs density was calculated as average number of TILs per DCIS ducts. We also counted the highest TILs around a single duct. The degree of circumferential TILs was estimated on a scale from 0 to 3 with the later showing complete cuffing of TILs around the ductal space. The mammographic images were reviewed and the span of calcifications was measured. The EB was reviewed and DCIS was graded from low to high. The pathologic and radiologic variables were correlated with HG-DCIS vs. others [no residual disease, invasive carcinoma (IC) or non-HG-DCIS].

Results: In a total of 129 DCIS cases, 26 (20.2%) had upgrade, 12 (9.3%) to IC, and 14 (10.9%) to HG DCIS. All cases with DCIS accompanying IC were low to intermediate grade. The median and range of the average number of TILs in cases which were upgraded to HG-DCIS was 4.5 (0 to 20) vs. 2 (0 to 16) for those without upgrade (p=0.004). The best cutoff was 3 cells with AUC of 0.732. The median and range of the highest number of TILs around a single duct in cases which were upgraded to HG-DCIS was 12 (1 to 118) vs. 6 (0 to 95) for those without HG-DCIS (p=0.016). The best cutoff was 9 cells with AUC of 0.699. All other variables did not correlate with upgrade. Circumferential and touching (average or highest number) TILs did not correlate with the overall upgrade to HG-DCIS and/or IC.

Conclusions: Touching TILs detected in low risk DCIS correlates with upgrade to HG-DCIS, but not with upgrade to IC. More studies with larger number of cases are warranted to examine our findings.

294 Significance of HER2 in Microinvasive Carcinoma of Breast: A Single Academic Institution Experience

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Disclosures: Huina Zhang: None; Ioana Moisini: None; Bradley Turner: None; Xi Wang: None; David Hicks: None

Background: It has been reported that HER2 overexpression/amplification is highly prevalent in the microinvasive carcinoma of breast compared with both invasive carcinoma (pT1a and above) and ductal carcinoma in situ (DCIS). However, the significance of HER2 in the microinvasive carcinoma has rarely been addressed. The aim of this study was to investigate whether HER2 positivity is associated with any specific clinicopathologic features and follow-up outcomes, in comparison to the HER2 negative counterparts.

Design: Cases with diagnosis of microinvasive carcinoma of breast were retrospectively identified between 2007 and 2019. The clinicopathologic characteristics (age, lymph node status, DCIS component, tumor infiltrating lymphocytes (TILs), hormonal receptors (HR), HER2 status and Ki-67) and follow-up information were collected and analyzed.

Results: Forty-two cases of microinvasive carcinoma with available HR and HER2 status were identified including 14 HER2 positive and 28 HER2 negative cases (23 HR+/HER2- and 5 HR-/HER2-). Neither patient age at diagnosis nor number of microinvasive carcinoma foci

showed significant difference between HER2 positive and negative groups (Table 1). The majority of microinvasive carcinoma cases were associated with a large volume of intermediate to high grade, solid and/or cribriform type of DCIS with central necrosis, regardless of HER2 status (Table 1). The presence of moderate to abundant amount of TILs and the Ki-67 expression were significantly increased in the HER2 positive cases (Table 1). Nodal metastasis was found in two cases (4.7%) and none of the HER2 positive cases had nodal metastasis. Clinical follow-up showed that the majority of patients with HER2 positive disease (78%, 11/14) did not receive HER2 targeted therapy. All 42 patients were alive without disease recurrence/distant metastasis, with an average follow up of 42 months.

Table 1: Clinicopathologic characteristics associated with HER expression in microinvasive carcinoma

	HER2 positive	HER2 negative	p-value
Age (years)	60.7	58.3	0.62
Multiple foci (%, n)	50% (7)	46% (13)	0.89
Average Size of DCIS (cm)	4.84	3.44	0.12
Presence of moderate to abundant TILs* (%)	**83**	**44**	**0.03**
Average Ki-67 (%)	**18.3**	**8**	**0.03**

* Tumor infiltrating lymphocytes

Conclusions: Similar to larger HER2 positive invasive breast carcinomas, HER2 positivity in microinvasive carcinoma is associated with triggering a host immune response and increased tumor cell proliferation. In contrast to HER2-positive invasive carcinomas of higher stage, HER2 overexpression in microinvasive carcinomas appears to not be associated with metastasis/recurrence compared to HER2 negative microinvasive disease in our series. Whether or not HER2-targeted therapy is warranted in this clinical setting merits additional study.

295 Immune Cells Subpopulations in Tumor Microenvironment and their Prognostic Value in Triple Negative Breast Cancer

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Background: Immune response mediates tumor progression and response to treatment, and the immune cell composition in tumor microenvironment is diverse including lymphocytes, macrophages, and other cell types. We have shown that tumor infiltrating lymphocytes (TILs), tumor infiltrating lymphocytes volume (TILV), and regulatory T cells (Tregs) have positive prognostic value in triple negative breast cancer (TNBC). In this study, we further evaluate the prognostic significance of B lymphocytes, plasma cells, tumor-associated macrophages (TAM), and CD4/CD8 ratio in triple negative breast cancer (TNBC).

Design: Tissue microarrays from fifty-eight TNBC patients who underwent neoadjuvant therapy (NACT) were prepared. Immunohistochemistry was performed to study the immune cell subpopulations. The statistic and Kaplan-Meier survival analysis were performed.

Results: CD163+ TAM, but not CD68+ TAM, are associated with smaller tumor size (p=0.02), higher pathologic complete response (pCR) rate (p=0.009), and better overall survival (p=0.03). CD20+ B lymphocytes correlate with smaller tumor size (p=0.007) and higher pCR rate (p=0.04). CD20+ B lymphocytes also correlate with lower stage and better overall survival but with no statistical significance (p=0.1 and p=0.07, respectively). CD4/CD8 ratio reversely correlates with pCR rate, but it has no association with tumor size, stage, or overall survival. High CD138+ plasma cells are associated with high pCR rate. TNBC cases with two or more favored parameters (TILV and/or TILs, CD163, Tregs or CD20) have much better overall survival (p=0.02).

Conclusions: TILV and/or TILs, Tregs, B lymphocytes, and CD163 TAM are predictive and prognostic biomarkers in TNBC microenvironment and can be used to predict the pCR rate and overall survival. Evaluation of these parameters will optimize risk stratification and therapeutic efficacy for TNBC patients. Large-scale studies are undergoing to validate these findings.

296 Is Conservative Management of Ductal Carcinoma-In-Situ Risky?

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Disclosures: Lan Zheng: None; Yesim Gokmen-Polar: None; Sunil Badve: None

Background: Ductal carcinoma in situ (DCIS) is a common form of non-invasive breast cancer. With the widespread adoption of population-based breast cancer screening, it represents approximately 20-25% of all new breast neoplastic lesions diagnosed. The natural history of DCIS is poorly understood and risk of DCIS progress to invasive carcinoma is remain unknown. The goal of this study is to determine the invasive cancer upstaging rates at excision for women diagnosed with DCIS on biopsy.

Design: Cases of DCIS diagnosed from 2010 to 2012 in out institution were respectively reviewed. Patients diagnosed with DCIS with synchronous invasive ductal carcinoma, DCIS with microinvasion or patient with no surgical excision were excluded. Patient demographic information including age, race, family history and imaging results were recorded. Surgical pathology reports were reviewed for upstaging to invasive disease.

Results: The study group included 258 patients with DCIS diagnosed by core needle biopsy. The median age at diagnosis of DCIS was 58.1 years (range, 29-90 years). 62.8% were Caucasian women and 16.7% were African American. Histologically, 45.3% of the case were high grade, 75.5% containing comedonecrosis, 82.1% were ER positive and 71.2% were PR positive. 16.7% patients underwent lumpectomy and 83.3% patients underwent mastectomy. The total upstage rate from DCIS to invasive disease was 15.5% (40/258): histologically 6.5% for low grade, 38.7% for intermediate grade and 54.8% for high grade DCIS. 24 patients had recurrent disease, among them 9 patients had recurrence of invasive carcinoma and 10 patients had recurrence of DCIS. There were 36 patients eligible for the COMET Trial, 34 for the LORIS Trial and 8 for the LORD Trial. The upgrade rate to an invasive carcinoma was 5.5% (2/36) for COMET, 5.8% (2/34) for LORIS and 0% (0/8) for LORD eligible cases (Table 1).

	Entire population (n=258) No. (%)	**COMET (n=36) No. (%)**	**LORIS (n=34) No. (%)**	**LORD (n=7) No. (%)**
Age (years, mean [range])				
	58.1 (29-90)	58.6 (43-86)	60.2 (46-86)	59.5 (46-86)
Race				
Caucasian	162 (62.8)	20 (55.5)	19 (55.9)	4 (57.1)
African-American	43 (17.9)	6 (16.7)	5 (14.7)	1 (14.3)
Not available	50 (19.3)	10 (27.8)	10 (29.4)	2 (28.6)
Hormone receptor status				
ER +/ER -	207 (82.1)/51 (17.9)	33 (91.6)/3 (8.4)	30 (88.2)/4 (11.8)	7 (100)/0
PR +/PR -	181 (71.2)/77 (28.8)	30 (83.3)/6 (16.7)	27 (79.4)/7 (20.6)	6 (85.7)/1 (14.3)
DCIS grade on core needle biopsy				
Low	16 (6.20)	6 (16.7)	5 (14.7)	7 (100)
Intermediate	122 (47.28)	30 (83.3)	29 (85.3)	0
High	117 (45.35)	0	0	0
Comedonecrosis				
	195 (75.6)	0	0	0
Upgrade to invasive carcinoma				
	31 (12.0)	2 (5.5)	2 (5.8)	0
Recurrent at 15 years in non-upgrade patients				
	9 (3.96)	2 (5.88)	2 (6.25)	0
RFS in upgrade patients (years, mean [range])				
	7.42 [0.12-9.16]	3.60 [0.53-5.33]	3.60 [0.53-5.33]	0
RFS in non-upgraded patients (years, mean [range])				
	6.28 [0.41-12.29]	6.38 [0.23-9.20]	6.74 [0.23-9.20]	6.06 [3.84-8.57]

Conclusions: The upgrade rate of the whole study is low (15.5%). Patients selected according to the criteria of COMET, LORIS and LORD showed significant decrease of the upgrade rate to invasive carcinoma. The result of this study may suggest that careful patient selection for DCIS active surveillance trails carries a low risk of missing occult invasive carcinoma.

297 Apocrine Variant of Pleomorphic Lobular Carcinoma In Situ (AP-LCIS): Further Clinical, Histopathological, Immunohistochemical and Molecular Characterization of an Emerging Entity

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Disclosures: Elaine Zhong: None; James Solomon: None; Esther Cheng: None; Jordan Baum: None; Wei Song: None; Syed Hoda: None

Background: AP-LCIS is distinguished by "pleomorphic" discohesive cells with (w/) abundant granular eosinophilic ("apocrine") cytoplasm & prominent nucleoli; & is usually accompanied by necrosis & calcifications (calcs). Although some features have been recently described (*Am J Surg Pathol* 2019;43:399-408, others), the entity is yet to be fully characterized.

Design: The departmental data were searched for AP-LCIS (excluding concomitant ductal carcinoma [ca]), over 10-years (7/2010-6/2019). All were E-cadherin (-) & p120 cytoplasmic (+). Immunohistochemistry (IHC) for ER, PR, HER2, Ki67, AR, & aurora kinase A (AURKA, a key regulator of cell cycle) was performed. AURKA was assessed for nuclear staining. Key slides were macrodissected & tested by the Oncomine Comprehensive Assay v2 (ThermoFisher, Waltham, MA).

Results: 11 cases of pure AP-LCIS, all females & none bilateral, were evaluated (**Figure 1**). Mean age: 64 (range 46-76). 9/11 (82%) presented w/ calcs on screening. 5 (45%) had necrosis, 9 (82%) had calcs. 7 (64%) had concomitant classic LCIS & 5 (45%) florid LCIS. **Table 1** & **Figure 1** show other IHC results. 9 (82%) were sequenced (**Figure 2**). Mean number of variants: 2.4. 8 had local excision, 1 mastectomy, 1 radiation, & 0 chemotherapy. In a mean followup of 57 mo, 2 AP-LCIS recurred & 1 had invasive (inv) lobular ca. AURKA showed 1-5% nuclear (+) in 3 (27%) of AP-LCIS & was not significantly associated w/ invasion or recurrence (Pearson correlation).

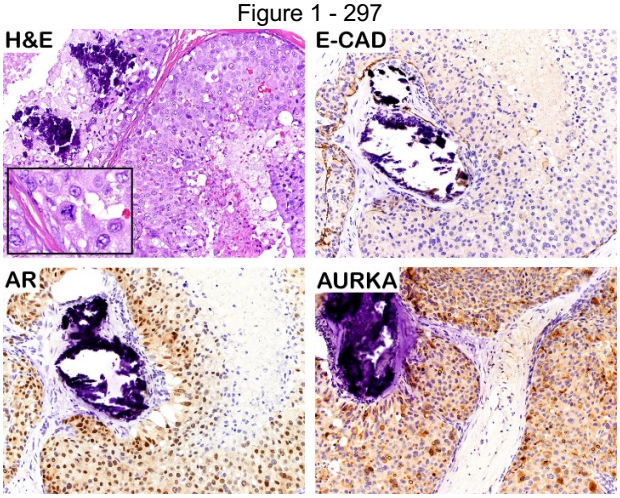
23 AP-LCIS w/ concurrent inv lobular ca (13 pleomorphic, 13 classic), all females & none bilateral, were also evaluated. Mean age: 66 (range: 49-83). 15 (65%) presented w/ calcs. 18 (78%) were sequenced. Mean number of variants: 3.3. 2 patients had known germline mutation present in tumor: 1 *BRCA1* & 1 *ATM*. There was no significant difference in IHC or mean number of variants of AP-LCIS w/ & w/o inv ca (Chi-square test, Student's t-test). AURKA(+) in 9 (43%) in this set of AP-LCIS was not significantly associated w/ nodal positivity (seen in 3 cases) (Pearson correlation). No patient had distant metastasis or died of disease.

Table 1. Immunohistochemical Profile of AP-LCIS

	AP-LCIS, all cases n: 34 (%)	**AP-LCIS, pure n: 11 (%)**	**AP-LCIS, associated with ILC n: 23 (%)**
E-cadherin (-)	**33/33**	11/11	22/22
p120 (+)*	**32/32**	11/11	21/21
ER (+)	**23/34 (68)**	9/11 (82)	14/23 (61)
PR (+)	**12/34 (35)**	5/11 (45)	7/23 (30)
HER2 (+)**	**9/34 (26)**	4/11 (36)	5/23 (22)
Ki67 >15%	**5/34 (15)**	0/11	5/23 (22)
AR (+)	**28/31 (90)**	10/10 (100)	18/21 (86)
AURKA (+)***	**12/32 (38)**	3/11 (27)	9/21 (43)

*: cytoplasmic; **: HER2+ by IHC (3/9), by FISH (1/4), 5/9 were 2+ by IHC & FISH was not performed; (+) for ER, PR, and AR: ≥1%; ***: >0% nuclear positivity.

Figure 1 - 297



Conclusions: In this cohort, AP-LCIS presented most often with calcs. AP-LCIS showed AR (+, in 90%), ER (-, 32%), HER2 (+, 26%), *CDH1* variants in 89%, *ERBB2* in 44%, and *PIK3CA* in 30%. AURKA (+, 38%) was not associated w/ outcome measures.

298 Genomic Profiling of Four Histological Subtypes of Metaplastic Breast Carcinoma Reveals Genetic Heterogeneity

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Disclosures: Siyuan Zhong: None; Shuling Zhou: None; Anqi Li: None; Hong Lv: None; Shaoxian Tang: None; Ruohong Shui: None; Yang Wentao: None

Background: Metaplastic breast carcinomas (MBCs) are a rare and histologically heterogenous group of breast cancers. Although most MBCs are triple negative, the clinicopathological features diverse among different subtypes. The histological diversity of MBC may be related to different genetic background of each subtype. To improve the understanding of biological characteristics of MBC, four histological subtypes of MBCs were analyzed.

Design: 22 MBCs, including ten squamous cell carcinomas (SCCs), three matrix-producing carcinomas (MPCs), four low grade fibromatosis-like spindle cell carcinomas (FLSCCs) and five moderate to high grade spindle cell carcinomas (SpCCs) were sequenced by DIAN (Hangzhou Lab) using a 324-gene platform (FoundationOneCDx) with licensed technologies. The results were compared with other triple-negative breast cancers in The Cancer Genome Atlas (TCGA) database.

Results: The results showed that SCCs had the highest mutation load among four subtypes(9-29 mutations/case, mean 14.7/case). Genes with high frequency mutations were *TP53* (9/10,90%),*PIK3CA* (7/10,70%),*MLL2*、*PTCH1*、*TSC1*、*STK11*、*SPEN*、*PTEN*(each gene3/10,30%) and *FGF3* amplification(3/10,30%). MPCs had high frequency mutations of *TP53*(3/3,100%), *BRCA1*(2/3,67%) and *CDKN2A* deletion(2/3,67%), *RAD21* amplification(2/3,67%). Hotspot mutations in *PIK3CA*(SpCCs4/5,80%, *FLSCCs* 3/4,75%) and *TERT* promoter (SpCCs4/5,80%, FLSCCs 3/4,75%) were found in spindle cell carcinoma including both SpCCs and FLSCCs. However, SpCCs also had a pathogenic mutation of *HRAS*(3/5,60%) and copy number variation of *MTAP* deletion(4/5,80%),*CDKN2B* deletion(4/5,80%)and *CDKN2A* deletion(4/5,80%), suggesting that the unique genetic alteration of SpCCs may lead to higher degree of malignancy than FLSCCs. Compared with the common mutated genes of triple-negative breast cancer in TCGA database(*TP53*153/171,89%, *PIK3CA*12/171,7%), mutation frequency of *TP53* in SpCCs(1/5,20%) and FLSCCs (0%) was much lower,while there was no *PIK3CA* mutation in MPCs. SpCCs(4/5,80%) and FLSCCs(3/4,75%) had more *TERT* promoter mutations than other triple-negative breast cancers.

Figure 1 - 298

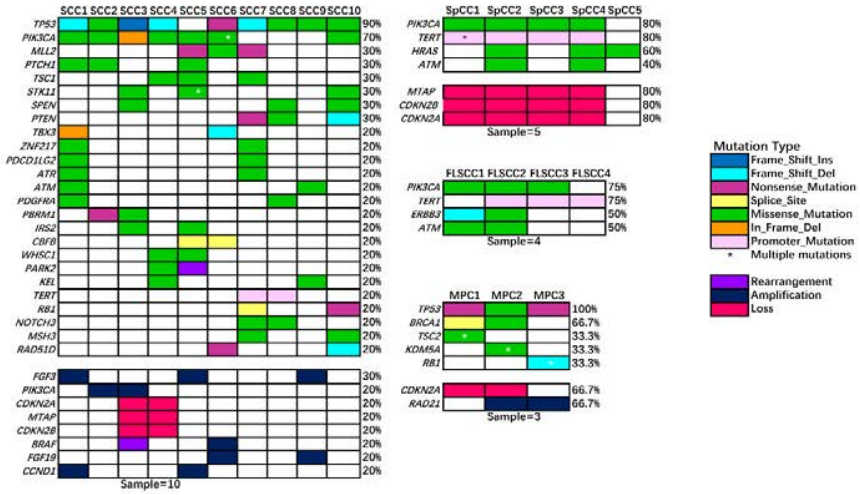
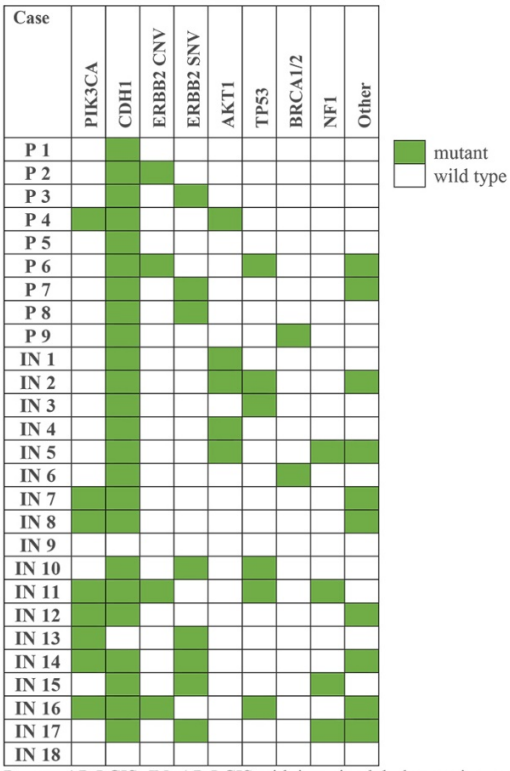


Figure.1 Mutant genes in four different subtypes of metaplastic breast cancers

Figure 2 - 297



P: pure AP-LCIS; IN: AP-LCIS with invasive lobular carcinoma; CNV: copy number variant;

SNV: single nucleotide variant

Conclusions: Our study reveals that the genetic changes of MBCs are significantly different from those of other triple-negative breast cancers. Different histological subtypes of MBCs have their own characteristics of genetic mutation. The histological and biological diversity may be related to its genetic heterogeneity.

299 The qRT-PCR-Based DNA Homologous Recombination Deficiency 4-Gene Score Predicts Pathologic Complete Response to Platinum-Based Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer

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Disclosures: Ke Zuo: None; Xiaoying Yuan: *Employee*, Shuwen Biotech Co., Ltd; Xizi Liang: None; Qi Lv: None; Yajing Zhao: *Employee*, Shuwen Biotech Co.,Ltd; Huanhuan Yang: None; Xingmin Li: *Employee*, Shuwen Biotech Co. LTD.; Wentao Yang: None

Background: Platinum-based neoadjuvant chemotherapy (NACT) improves pathologic complete response (pCR) in triple-negative breast cancer (TNBC). It would be helpful to personalize the use of platinum agents if a predictive biomarker for platinum sensitivity could be developed. However, these data are limited. Previous studies showed DNA homologous recombination deficiency (HRD) was a potential biomarker predicting pCR in ER-negative breast cancer. Therefore, we try to develop a HRD gene expression score to predict the tumor sensitivity to platinum-based NACT in TNBC.

Design: A retrospective cohort of 127 TNBC patients from 2012 to 2017 were included in this study. All of them were diagnosed and received platinum-based NACT in Fudan University Shanghai Cancer Center. Clinical data and pathological data of the patients were collected and reviewed. By using quantitative reverse transcription-polymerase chain reaction (qRT-PCR), the HRD associated gene expression level, e.g. RAD51, XRCC5, RIF1, PARPBP, PARP1, BRCA1 etc, were analyzed from the formalin-fixed paraffin-embedded core needle biopsy samples which obtained before NACT. A random forest model was built to estimate the weight of each gene expression level and clinical-pathological factors. Samples were randomized into the training set and validation set with different splitting percentage from 50%:50% to 90%:10%. The training set was used to modulate parameters and select the best model using 5-fold cross validation. The performance of the final model was evaluated in the validation set.

Results: A 4-gene (BRCA1, XRCC5, PARP1, RAD51) expression signature scoring system was developed. Higher score TNBC had nearly quadruple likelihood to achieve pCR to platinum-based NACT compared with a lower score [odds ratio(OR)=3.878; P<0.001]. At the cut-off value of -2.644, the 4-gene score system showed high sensitivity for predicting pCR in breast (93.0%) and pCR in both breast/axilla (91.8%), while, at the cut-off value of -1.969, the 4-gene score system showed high specificity for predicting pCR in breast (85.7%) and pCR in both breast/axilla (80.8%) (Table 1). Additionally, Ki-67≥40% (OR=4.569; P=0.028) and N stage(OR=2.279; P=0.009) were also independent predictors of pCR. The 4-gene score was positively correlated with Ki-67≥40% (P=0.002), but negatively correlated with positive lymph nodes counts (P=0.003) .

Table 1 Performance of 4-gene score in predicting pCR breast and pCR breast/axilla

	pCR in breast				pCR in both breast/axilla			
Cut-off	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
-2.644	93.0%	57.1%	63.8%	90.9%	91.8%	51.3%	54.2%	90.9%
-1.969	64.9%	85.7%	78.7%	75.0%	65.3%	80.8%	68.1%	78.8%

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; pCR, pathological complete response.

Conclusions: The qRT-PCR-based 4-gene score is an effective predictor of pCR to platinum-based NACT in TNBC.