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121 Actionable Genetic Alterations in Advanced Breast Cancer: A Frequency Analysis by Molecular Subtype

Sara E Abbott[1], *Russell Broaddus*[2], *Hui Chen*[1]. [1]UT MD Anderson Cancer Center, Houston, TX, [2]M.D. Anderson Cancer Center, Houston, TX

Background: When standard breast cancer (BC) treatment options are no longer effective, the identification of alternative actionable gene targets may help inform the next best option for patient care. We present an analysis of a unique population comprised exclusively of patients with advanced BC presenting to a large tertiary care cancer center in order to determine the frequency and distribution of genetic alterations with potential therapeutic targets.

Design: We included all patients (n=83) who had 134-gene panel next generation sequencing (NGS) performed from 2016-2017 to help guide clinical decision making in the setting of treatment resistant advanced BC. Metastatic/recurrent tumor was preferentially used for testing (n=66). When not available, primary tumor was used (n=17). Initial review identified any alteration present in a clinically actionable gene, defined as a gene that can be therapeutically targeted.

Results: Forty-nine patients (59%) were found to have a genetic alteration in at least one actionable gene, most of which (29/49) had an alteration in only one gene (Table 1). Patients with ER+/HER2- BC were most likely to have an alteration identified in an actionable gene (73% vs 54% in HER2+ and 43% in TNBC; p=0.045). Furthermore, the alterations identified in ER+/HER2- BCs occurred across a broader range of actionable genes (n=22) then did those identified in TNBCs and HER2+ BCs (n=9 and n=8, respectively). Mutations were more common (62 events in 20 genes) than amplifications (24 events in 9 genes). The most common mutations identified in actionable genes were in *PIK3CA* (27%), *ATM* (6%), *NF1* (6%), and *PTEN* (6%), and the most common amplifications occurred in *CCND1* (11%) and *FGFR1* (6%) (Table 2). Comparison between the BC subtypes revealed that ER+/HER2- BCs were more likely to have *CCND1* amplification than TNBCs (18% vs 0%) and also trended toward having more *PTEN* mutations and *FGFR1* amplifications than TNBC (13% vs 0% for both.)

Table 1. Frequency of alterations in actionable genes by breast cancer subtype

	Total N = 83 (100%)	ER+/HER2- N = 40 (48%)	TNBC N = 30 (36%)	HER2+ N = 13 (16%)	P-value
Alteration in any actionable gene	**49 (59%)**	**29 (73%)**	**13 (43%)**	**7 (54%)**	**0.045**
1 gene	29 (35%)	15 (38%)	10 (33%)	4 (30%)	-
2 genes	8 (10%)	6 (15%)	0	2 (15%)	**0.044**
≥ 3 genes	12 (14%)	8 (20%)	3 (10%)	1 (8%)	-
Number of unique actionable genes with alterations	27	22	9	8	

Table 2. Most common alterations in actionable genes by breast cancer subtype

	Total Patients N = 83 (100%)	ER+/HER2- N = 40 (48%)	TNBC N = 30 (36%)	HER2+ N = 13 (16%)	P-value
Somatic mutation					
Oncogenes					
PIK3CA	22 (27%)	14 (35%)	7 (23%)	1 (8%)	-
EGFR	2 (2%)	1 (3%)	1 (3%)	0	-
ERBB2	2 (2%)	1 (3%)	1 (3%)	0	-
ERBB4	2 (2%)	2 (5%)	0	0	-
KRAS	2 (2%)	1 (3%)	1 (3%)	0	-
Tumor suppressor genes					
ATM	5 (6%)	4 (10%)	0	1 (8%)	-
NF1	5 (6%)	2 (5%)	3 (10%)	0	-
PTEN	5 (6%)	5 (13%)	0	0	0.076
PIK3R1	3 (4%)	1 (3%)	0	2 (15%)	-
BRCA1	2 (2%)	1 (3%)	1 (3%)	0	-
BRCA2	2 (2%)	0	2 (7%)	0	-
Amplification					
CCND1	9 (11%)	7 (18%)	0	2 (15%)	**0.023**
FGFR1	5 (6%)	5 (13%)	0	0	0.076
CCNE1	2 (2%)	0	1 (3%)	1 (8%)	-
MDM2	2 (2%)	1 (3%)	0	1 (8%)	-
PIK3CA	2 (2%)	0	1 (3%)	1 (8%)	-

Conclusions: Although most common in ER+/HER2- BC, alterations in actionable genes occur frequently in all BC subtypes. With few exceptions, the alterations occur at low frequencies across a wide number of genes. Therefore, the composition of an NGS panel used for testing should reflect this finding. Additional analysis to determine which patients were ultimately able to receive targeted therapy as a result of their specific alteration(s) is being performed and will further inform the utility of this testing approach in advanced BC.

122 ESR1 Somatic Mutation Analysis in Advanced Breast Cancer: Correlation with Clinicopathologic and Molecular Features

Sara E Abbott[1], *Russell Broaddus*[2], *Lei Huo*[3], *Constance Albarracin*[1], *Mark Routbort*[4], *Keyur Patel*[5], *Rajyalakshmi Luthra*[1], *Hui Chen*[1]. [1]UT MD Anderson Cancer Center, Houston, TX, [2]M.D. Anderson Cancer Center, Houston, TX, [3]UT MD Anderson Cancer Center, [4]Bellaire, TX, [5]UT-MD Anderson Cancer Center, Sugar Land, TX

Background: Endocrine therapy resistance is a major challenge in the treatment of ER+ breast cancer (BC). The subset of affected patients (~25%) cannot be easily identified until treatment failure occurs. Emerging data suggest somatic mutations in the ligand-binding domain (LBD) of *ESR1*, reported to occur in 0.5 to 21% of ER+ BCs, may lead to endocrine therapy resistance via constitutive activation of ER. Characterization of the clinicopathologic and molecular features of *ESR1* mutated BCs may aid in the early identification of patients at risk for a more aggressive disease course.

Design: Eighty-four patients with advanced BC (51 ER+, 31 triple negative, 10 HER2+) who had a next generation sequencing 134-gene panel performed from 2016-2017 were studied. *ESR1* mutation status was examined with respect to clinical, pathologic, and molecular variables.

Results: Twelve patients (14%) each had a single *ESR1* mutation in the LBD hotspot. *ESR1* mutations occurred only in patients with ER+ BC and nearly all (11/12) were identified in recurrent/metastatic disease specimens (Table 1). There was no association between *ESR1* mutation and HER2 status or tumor histology. While a minority of ER+ cases (4/48) were found to have loss of ER expression by IHC at ≥ 1 site of recurrence/metastasis in BC cases without *ESR1* mutation, all cases with *ESR1* mutation showed preserved ER expression across all sites and time points of sampled recurrent/metastatic disease (Table 2). A concurrent *TP53* mutation was less likely in BCs with *ESR1* mutation than those without (17% vs 67%, p<0.01), a trend that continued when only ER+ BCs were compared. Progression free survival (PFS) amongst ER+ patients showed that those with *ESR1* mutations trended toward a longer PFS (median 44.5 vs 26.9 months) though multiple recurrences at frequent intervals were common thereafter. Of note, one patient had 2 samples tested; *ESR1* mutation was not found in locally recurrent tumor, but was detected in a distant metastasis that occurred 2 years later.

Table 1: Advanced breast cancer (n=84)

	ESR1 somatic mutation present (n=12)	*ESR1* somatic mutation absent (n=72)	P-value
Age (years), median (range)	46 (31-57)	49 (23-73)	0.10
ER status by IHC, n (%)			**<0.01**
ER+	12 (100%)	39 (54%)	
ER-	0	33 (46%)	
Biomarker status, n (%)			**<0.01**
ER- HER2-	0	31 (43%)	
ER- HER2+	0	2 (3%)	
ER+ HER2-	9 (75%)	31 (43%)	
ER+ HER2+	3 (25%)	8 (11%)	
Histologic type, n (%)			0.45
Ductal	9 (75%)	52 (72%)	
Lobular	2 (17%)	6 (8%)	
Other	1 (8%)	14 (19%)	
Tested tissue site, n (%)			**<0.01**
Primary tumor	1 (8%)	17 (24%)	
Recurrence/Metastasis	11 (92%)	55 (76%)	
***TP53* somatic mutation, n (%)**			**<0.01**
Present	2 (17%)	48 (67%)	
Absent	10 (83%)	24 (33%)	

Table 2: ER+ advanced breast cancer (n=51)

	ESR1 somatic mutation present (n=12)	*ESR1* somatic mutation absent (n=72)	P-value
Tested tissue site, n (%)			1
Primary tumor	1 (8%)	6 (15%)	
Recurrence/Metastasis	11 (92%)	33 (85%)	
***TP53* somatic mutation, n (%)**			0.09
Present	2 (17%)	18 (46%)	
Absent	10 (83%)	21 (54%)	
ER discordance between primary tumor and recurrent/metastatic disease, n (%)			0.56
ER expression preserved	12 (100%)	32 (82%)	
ER loss observed	0	4 (10%)	
No repeat ER testing available	0	3 (8%)	
Progression free survival (mos), median (range)	44.5 (5.1-346.2)	26.9 (1.5-158.3)	0.11

Conclusions: We present one of the largest cohorts of ESR1 mutated BC patients identified to date. *ESR1* mutations were exclusively present in ER+ BC with preserved ER expression. Patients followed an aggressive clinical course with delayed, but frequent disease progression. Screening for *ESR1* mutations is recommended early in disease progression using the metastatic/recurrent tumor, particularly when ER expression is preserved, in order to identify patients at risk for endocrine therapy resistance.

123 Mutational and Immune Profiling of Metaplastic Breast Cancer: Correlation with Survival

Michelle Afkhami[1], *Daniel B Schmolze*[1], *Susan E Yost*[2], *Paul H Frankel*[3], *Andy Dagis*[2], *Carrie Louie*[2], *Raju K Pillai*[3], *Patricia Aoun*[1], *Yuan Yuan*[2]. [1]City of Hope National Medical Center, Duarte, CA, [2]City of Hope National Medical Center, [3]City of Hope Medical Center, Duarte, CA

Background: Metaplastic breast cancer (MpBC) is a rare malignancy with aggressive clinical presentation and poor prognosis. The goal of this study is to characterize the mutational landscape and immune microenvironment of MpBC and to correlate with clinicopathologic characteristics.

Design: We identified 23 patients diagnosed with MpBC between 1995 and 2013. A next generation sequencing (NGS) based mutational assay targeting 48 genes was performed on 21 patients.

Clinicopathologic characteristics were captured including age, race, stage, chemotherapy and radiation therapy history, 5-year relapse free survival (RFS), progression free survival (PFS), and overall survival (OS). Immunohistochemistry (IHC) for CD3, CD4, CD8, and PDL1 (Ventana clone SP263) was performed on 18 samples.

Results: Three specimens failed to yield results, and one patient had no survival data. The median age at time of diagnosis was 65.5 years (range 35-84). The subtypes of MpBC were: 7 squamous (35%), 6 spindle (30%), 3 mixed squamous and spindle (15%) and 4 mesenchymal (20%). A total of 14 (70%) received chemotherapy, and 13 (65%) received radiation therapy. The median follow-up time for surviving patients was 5.35 years (range 0.09 – 10.6). Median RFS was 5.23 years (95% CI 1.13-), and OS was 5.23 years (95% CI 2.08-). The most commonly mutated genes were TP53 (65%), PIK3CA (45%), PTEN (15%). For PIK3CA, RFS at 2 years was 33% (95% CI 0.03-0.64) for patients with mutations, versus 100% for those without mutations ($p < 0.01$, log-rank). OS at 2 years was 56% for those with mutations (95% CI 0.23-0.88), versus 100% for those without mutations ($p < 0.02$). RFS at 5 years was 0.56 (95% CI 0.20-0.80) for those with PIK3CA mutations, and 0.89 (95% CI 0.43, 0.98) for those without (p=0.007). Overall survival was 0.30 (95% CI 0.05, 0.61) at 5 years in patients with PIK3CA mutations compared with 0.89 (95% CI 0.43, 0.98) without PIK3CA mutations (p=0.008). There was no assocuation between TP53 or PTEN mutations and survival. In 66% of the cases a majority of tumor infiltrating lymphocytes were CD8 positive. PDL1 expression was seen in 33% of samples. PDL1 expression was associated with poor RFS (HR 1.218, p=0.05) and overall survival (HR 1.227, p = 0.04). There was no correlation between CD3, CD4 or CD8 TILs quantification and survival.

Conclusions: PIK3CA mutation and PDL1 expression confers poor prognosis in this small cohort of patients with MpBC. A larger cohort of patients is needed to verify these findings.

124 Title: Histologic and Molecular Subtype of CHEK2 and PALB2 Associated Breast Cancers

Indu Agarwal[1], *Megan E Sullivan*[2]. [1]NorthShore University Health System, Evanston, IL, [2]NorthShore University Healthsystem, Evanston, IL

Background: Although the most widely known, *BRCA* mutations are only responsible for 10–20% of cases of breast cancer in patients with early-onset or a family history. With expanded genetic testing, multiple breast cancer predisposing genes have been identified, including CHEK2 and PALB2. Compared to the *BRCA* associated carcinomas, less is known about the histology found in patients with other high risk mutations. In this retrospective study, we identified women with either CHEK2 or PALB2 mutations who developed breast cancer and performed immunohistochemical stains to further subtype the tumors.

Design: Twenty women with mutations in *CHEK2 or PALB2* and a personal history of breast cancer were identified. Clinicopathologic data was collected including the number and type of breast cancers for each patient. Slides were available for review on 13 patients and Ki-67 immunohistochemistry (IHC) was performed on all invasive carcinomas (IC). 500 cells were counted to determine the Ki-67 index. Based on the IHC results, the ICs were categorized as: luminal A (ER/PR positive; HER2 negative; Ki-67 <14%), luminal B (ER/PR positive; HER2 negative; Ki-67 >14%), HER2 positive or triple negative (TN).

Results: 13 women with different germline CHEK2 mutations were identified. Of these, 8 developed IC, one with two separate ICs. Five had DCIS only. 6 (67%) of the ICs were ductal and 3 (33%) were lobular. 7 women with different germline PALB2 mutations were identified. All developed ICs, and two had multiple ICs for a total of 11 ICs. 9 (81%) of the ICs were ductal and 2 (18%) were lobular. There was no correlation between a specific mutation variant and the development of IC vs. DCIS or IDC vs. ILC. Tumor markers were available on 18 ICs, the majority of which luminal type. No HER2 positive cases were identified and only 1 PALB2 patient developed a TN tumor. Ki-67 was performed on all the ICs with available blocks (N=10) to further subclassify the luminal tumors (Table 1).

Mutation	Patients	Separate ICs	Luminal	Luminal A (<14%)	Luminal B (>14%)
CHEK2	13	9	9/9 (100%)	1/4 (25%)	3/4 (75%)
PALB2	7	11	8/9 (88%)	1/6 (17%)	5/6 (83%)

Conclusions: 38% of CHEK2 patients were diagnosed with DCIS compared with none in the PALB2 group who all presented with ICs. There was no correlation between the mutation variant in either group and the type of breast cancer. Overall, 19 of 20 (95%) total ICs in CHEK2 and PALB2 patients at our institution were ER/PR positive, HER2 negative. However, 80% had a proliferative index >14%, consistent with the more aggressive luminal B subtype.

125 PREVIOUSLY PUBLISHED

126 Implementation of a platform for objective immunohistochemistry analysis in a tertiary hospital from Chile: a pilot study

Viviana Ahumada[1], *Marcela Gallegos*[1], *Marcela Schultz*[2], *Daniel E Carvajal-Hausdorf*[3]. [1]Clinica Alemana - Facultad de Medicina Universidad del Desarrollo, [2]Santiago, [3]Clinica Alemana - Facultad de Medicina Universidad del Desarrollo, Santiago, Region Metropolitana

Background: Accurate and reproducible examination of predictive immunohistochemistry (IHC) assays is key for treatment selection in breast cancer. Local, conventional evaluation of IHC is subject to assay variability and interpretative subjectivity. Here, we applied an objective, automated platform for IHC quantification in a series of breast cancer biopsies and correlated its performance with traditional pathology assessment.

Design: IHC slides for ER, HER2 and Ki-67 from 32 core biopsies and 1 mastectomy diagnosed as ductal or lobular infiltrating carcinoma at a Chilean tertiary hospital were scanned using an Aperio® AT2 scanner (Leica). Whole tissue (WT) and tumor only (TO) were selected by an operator trained by a breast pathologist. These areas were analyzed using FDA-approved algorithms for nuclear and membrane positive pixel counting with capability for tumor cell detection. We compared agreement of WT and TO analysis to pathologist evaluation (PE) for percentage cell positivity (ER and Ki-67) and intensity scoring (HER2) using intraclass correlation (ICC) and kappa ( ) coefficients, respectively, as well as time required for analysis. All tests were two-sided (mean ± SEM).

Results: For all biomarkers, WT included an increased number of cells in the analysis, compared to strict TO selection (160,157 ± 14,124 vs 71,173 ± 9498, $P<0.0001$). For ER, TO and PE showed the highest concordance (ICC=0.93), while WT and PE had the lowest (ICC=0.81). For Ki-67, WT and TO showed the highest concordance (ICC=0.95), while WT and PE showed the lowest (ICC=0.8). When intensity scoring for HER2 was evaluated taking PE as standard, agreement was fair and similar for WT and TO (=0.36 and 0.37, respectively). Automated TO evaluation required an increased amount of time per case, compared to WT and PE (104.4 ± 9.1 vs 4.5 ± 0.1 vs 7.2 ± 0.4 minutes, respectively, $P<0.0001$).

Conclusions: While objective IHC analysis shows promising results when compared to standard evaluation, issues with tissue recognition and duration of analysis need to be solved. These methodologies might find their best application in classifying borderline cases.

127 Comparison of Clinicopathological Features between Hormone Receptor Positive and Negative Cases in HER2-Positive Breast Cancer Patients

Momoko Akashi[1], *Rin Yamaguchi*[2], *Hironori Kusano*[1], *Maki Tanaka*[3], *Hirohisa Yano*[1]. [1]Kurume University School of Medicine, Kurume, Fukuoka, [2]Kurume Univ/Medicine, Kurume, Japan, [3] Japan Community Health Care Organization Kurume General Hospital

Background: HER2-positive breast cancer (HER2+ BC) is often treated alike, but can be divided into luminal B (HER2+) type (LH) and HER2+ (non-luminal) type (NLH) by clinicopathological definition. Although HER2+ BC generally responds well to anti-HER2 therapy, some clinical reports describe a different treatment response depending on the intrinsic molecular subtype, or the expression level of hormone receptors in the LH group. However, few reports describe the difference from a pathological perspective. Thus, we reviewed the clinicopathological features of LH and NLH types in HER2+ BC patients.

Design: Patients with HER2+ BC were identified from 1301 patients who had surgical treatment for invasive breast cancer between January 2013 and December 2016. HER2+ BC was divided into two groups of LH or NLH, and the LH group was further divided into subtype groups expressing hormone receptors at high levels (LH-high) (Allred score 4-8) or low levels (LH-low) (Allred score 0, 2, 3). The following pathological findings were reviewed for each group: histological grading (HG), comedo necrosis, intraductal lesion, healing, fibrotic focus (FF), infarction and tumor-infiltrating lymphocytes (TIL) (high: >67%).

Results: HER+ BC was found in 218 patients (HER2 positive rate 16.8%). Excluding those treated by neo-adjuvant therapy, there were 169 patients with HER2+ BC (13%), of which 112 (66.3%) were in the LH group [91 (53.8%) in the LH-high and 21 (12.4%) in the LH-low groups] and 57 (33.7%) were in the NLH group. All groups presented high-HG (58.2% vs. 71.4% vs. 77.2%, $P=0.17$). Compared with the LH-high group, patients in the LH-low and NLH groups had significantly higher rates of comedo necrosis (67% vs. 90.5% vs. 87.7%, $P=0.003$), and adversely low FF (39.6% vs. 28.6% vs. 10.5%, $P<0.001$). Healing was notable in the LH-low and NLH groups with higher TIL level [healing (18.7% vs. 66.7% vs. 70.2%, $P<0.001$), high TIL (4.3% vs. 42.9% vs. 38.6%, $P<0.01$)]. No significant difference was observed in other features reviewed.

Conclusions: The LH group has two subtypes of LH-high and -low, and the latter group is characteristically closer to the NLH group than to the LH-high group in the HER2+ BC patients.

128 Androgen Receptor Expression In BRCA-Associated Breast Cancers

Constance Albarracin[1], *Celestine Marie Trinidad*[2], *Juhee Song*[3], *Kristin Ward*[4], *Sagar Dhamne*[5], *Daniel Rosen*[5], *Yun Wu*[6], *Hui Chen*[1], *Hayriye Sema Baghaki*[2], *Angelica M Gutierrez Barrera*[2], *Banu Arun*[7]. [1]UT MD Anderson Cancer Center, Houston, TX, [2]University of Texas MD Anderson Cancer Center, Houston, TX, [3]M.D. Anderson Cancer Center, [4]Texas Tech University Health Sciences Center at El Paso, El Paso, TX, [5]Baylor College of Medicine, [6]Houston, TX, [7]University of Texas MD Anderson Cancer Center

Background: More than 50% of hereditary breast cancers are due to known mutations in the breast cancer susceptibility genes BRCA1 and BRCA2. These tumors tend to be triple negative (TN) and treatment options are limited. Recently, therapies targeting the androgen receptor (AR) in breast cancer have been promising. However, little is known about its role in BRCA-associated cancers and its utility as a prognostic marker. Our goal is to characterize AR expression in these patients and its effect on recurrence and survival.

Design: The data set included 83 records on 76 unique patients. The analysis included 38 high risk patients who underwent genetic testing (10 BRCA1, 6 BRCA2, 22 BRCA negative), and 32 breast cancer cases not fulfilling the criteria for genetic testing were included to serve as sporadic controls. AR status was not available for 6 patients and were excluded. Scoring of ER, PR and HER2 were done as per ASCO/CAP guidelines. Tissue microarrays were prepared and immunohistochemically stained for AR. The samples were scored positive for AR when at least 10% of the nuclei showed positive expression. Two sample t-test and Fisher's exact test were used for statistical analysis. Kaplan-Meier method was used for analysis of overall (OS) and recurrence-free survival (RFS).

Results: BRCA1 and BRCA2 groups had a high nuclear grade in 70% and 83%, respectively. BRCA1 tumors were ER-neg in 80%, PR and HER2-neg in 100%, AR-neg in 90%. BRCA2 tumors were ER-neg in 67%, PR-neg in 83%, HER2-neg in 100%, and AR-neg in 100%. OS was significantly poorer in BRCA1 patients (p=0.0340) and higher stage (p=0.0012). AR was positive in 14% (10/70). AR-pos tumors had significantly lower nuclear grades (p=0.03). Eighty percent (8/10) were BRCA-neg and 30% (3/10) were TN. AR-pos group had a hazard ratio greater than one (HR=1.354) compared to the AR-neg group, however OS and RFS between groups were not significantly different.

Conclusions: In our study, BRCA-associated breast cancers are most likely to be negative for ER, PR, Her2, and AR. OS in these patients is strongly associated with BRCA1 mutation and stage but not with AR status. However, in terms of hazard ratios, AR-positive groups would have worse outcomes compared to the AR-negative group, though the difference between the two groups is not statistically significant. This shows the possible clinical value of AR as a marker for worse prognosis in high risk breast cancers. Further studies on larger patient numbers to validate these results are in progress.

129 Solid papillary carcinoma with reverse polarity of the breast displays morphologic, immunohistochemical and molecular characteristics in comparison to other benign or malignant papillary lesions of the breast. A comparative study of nine additional cases

Nadjla Alsadoun[1], *Gaetan Macgrogan*[2], *Caroline Trunzer*, *Magali Lacroix-Triki*[3], *Isabelle Bedgedjian*[4], *Elsy El Alam*[5], *Romain Boidot*[6], *Laurent Arnould*[7]. [1]Centre Georges François Leclerc, Dijon, Burgundy, [2]Bordeaux Aquitaine, [3]Institut Gustave Roussy, [4]Besançon Pathologie, [5]Institut Curie, Saint Cloud, IDF, [6]Centre Georges François Leclerc, [7]Centre G.F. LeClerc, Dijon, France

Background: Solid papillary carcinoma with reverse polarity (SPCRP), initially named breast tumor resembling the tall cell variant of thyroid papillary carcinoma is a rare breast cancer of favorable prognosis. This tumor belongs to the group of papillary mammary lesions and can be difficult to diagnose. We report here nine additional cases and perform a comparative study with three other types of papillary lesions of the breast in order to find characteristics that can help pathologists in routine practice.

Design: In this study, we describe its morphologic, immunohistochemical and molecular profiles in comparison to other breast papillary and micropapillary tumors, benign or malignant, as intraductal papilloma with usual ductal hyperplasia (IDPUDH), encapsulated papillary carcinoma (EPC) and invasive micropapillary carcinoma (IMPC).

Results: We studied nine SPCRPs and found that they harbor specific morphologic features as cuboid or tall cells with abundant eosinophilic cytoplasms located at the basal pole giving the impression of reverse nuclear polarity. Nuclei were of low grade, sometimes grooved. Immunohistochemistry (IHC) demonstrated the lack of myoepithelial cells along their fibrovascular stalks, as in EPC, questioning their invasive nature. SPCRPs showed a low Ki67 proliferative index, expression of CK5/6 as in IDPUDH, expression of calretinin and a low or lack of hormonal receptor (HR) expression that were not observed in other papillary and micropapillary breast tumors. By whole exome analysis, seven of nine SPCRPs (78%) harbored a hotspot mutation in *IDH2* (R172) that was totally absent in the six EPC, the six IMPC and the six IDPUDH. We also demonstrated for the first time in this tumor, that this specific and diagnostic *IDH2* mutation can be highlighted by IHC using a specific antibody (IDH1/2 mutant R132/R172). Six of nine SPCRPs (67%) also harbored *PRUNE2* mutation, including the two wild-type *IDH2* cases. Currently, these mutations are very unusual in other breast tumors. Moreover, transcriptomic analysis of SPCRP showed that Glypican 1 pathway was significantly enriched.

Conclusions: Our findings support the fact that SPCRP is a singular breast neoplasm, harboring specific morphologic, immunohistochemical and molecular features that distinguished them from other breast papillary and micropapillary tumors. Thus, morphologic features, IHC (CK5/6, HR, myoepithelial markers, calretinin and IDH2) as well as molecular analyses may easily separate it from the other differential diagnoses.

130 Myoepithelial Carcinoma of the Breast. A Clinicopathologic Study of 22 Cases.

Isabel Alvarado-Cabrero[1], *Raquel Valencia-Cedillo*[2]. [1]Mexican Oncology Hospital, Mexico, [2]Hospital de Oncología, CMN Siglo XXI IMSS, Cd Mexico, FDM

Background: Myoepithelial Carcinoma (MEC) of the breast is a rare malignant neoplasm composed entirely of myoepithelial cells. It consists of spindle and/or epithelioid cells with high mitotic activity and cytologic atypia. MEC can mimic several benign and malignant lesions and may be misdiagnosed by the pathologist.

Design: Twenty-two cases of MEC arising in the breast were retrieved from the consultation and pathology files at our Institution, covering a period of 12 years (2000-2012). Clinicopathologic features and immunophenotypes of these cases were evaluated.

Results: The patient´s age ranged from 39 to 76 years (mean 66 years) and 1 patient was male. the most common presentation was a unilateral palpable breast mass. The tumor size measured between 2.5 and 5.4 cm (mean 3.4 cm).

Grossly, the masses were unencapsulated but well demarked (6 cases) or ill-defined (16 cases). Cut surface revealed yellow-tan and white tumors, some of them with necrosis.

Microscopically, carcinomatous transformation in adenomyoepithelioma was seen in 3 cases. In most cases, the invasive tumor often proliferated around normal duct and lobules incorporating them into the lesion. Most tumors were composed enterely by an overgrowth of spindle cells, but 2 tumors consisted of epithelioid myoepithelial cells with large vesicular nuclei. The number of mitotic figures ranged from 0 to 10/10 high power field.

Immunohistochemical studies showed that the infiltrated cells were positive to p63, calponin, CK5/6 and Maspin. ER, PR and HER2 were negative in all 22 cases.

All patients underwent radical mastectomy and pathologic tumor stage included T2 (n:20) and T3 (n:2). All lymph nodes were negative for carcinoma.

Follow-up was available in twenty cases (mean 48 months, range:11 to 58 months). two patients had local recurrence, and six developed lung metastases.

At last contact three patients died of disease.

Conclusions: Myoeptihelial Carcinoma (MEC) of the breast is a rare malignant neoplasia

Due to the small number of cases presented in the literature, there is still relatively little known about the clinical course and prognosis.

MEC is a potentially aggressive malignant neoplasm

131 Extent of Metaplastic Differentiation in Metaplastic Mammary Carcinoma May Impact Tumor Stage and Behavior

Rebeca Alvarez[1], *Joshua Harford*[2], *Sheryl Jordan*[2], *Siobhan M O'Connor*[3], *Johann Hertel*[4]. [1]Durham, NC, [2]University of North Carolina at Chapel Hill, [3]University of North Carolina, Chapel Hill, NC, [4]University of North Carolina at Chapel Hill, Chapel Hill, NC

Background: Metaplastic carcinoma is a rare, aggressive subtype of invasive mammary carcinoma defined by the World Health Organization as "neoplasms characterized by differentiation of the neoplastic epithelium into squamous cells and/or mesenchymal-

looking elements." Previous studies have suggested that metaplastic carcinomas are aggressive carcinomas with a predilection for hematogenous spread. However, despite the significance of the diagnosis, there are no standardized criteria for diagnosis. In this study we reviewed all available cases to determine whether extent of metaplastic differentiation predicts tumor behavior.

Design: We reviewed our files for all metaplastic carcinomas between 2002 and 2014 and retrieved 22 cases with slides available for review from resection specimens at UNC-CH to determine the extent of metaplastic differentiation. The electronic medical record was also reviewed to determine tumor stage at the time of definitive surgical management.

Results: We found that tumors were either predominated by the metaplastic component (11 with > 80% metaplastic differentiation) or the by the epithelial component (9 with < 30% metaplastic differentiation); a minority show a balanced proportion (2 with 60% metaplastic differentiation). The metaplastic predominant tumors presented with a larger mean tumor size (51 mm) and lower N stage (2 of 11 with nodal involvement; both with N1); the epithelial predominant tumors presented with a smaller mean tumor size (33 mm) and higher N state (3 of 9 with lymph node involvement; two with N3). Nodal involvement was an important predictor of disease progression: 5 of 7 patients with nodal involvement dead of disease, whereas 2 of 15 with negative nodes at staging dead of disease.

Conclusions: Metaplastic carcinomas are a rare and aggressive subtype of invasive mammary carcinoma with implications for prognosis and treatment. Despite the clinical significance, the diagnosis lacks a consistent definition. We reviewed 22 cases of metaplastic carcinoma to evaluate whether extent of metaplastic differentiation predicts tumor behavior. We found that extensive metaplastic differentiation tends to predict a larger tumor volume but may be less lymph node involvement. Patients with predominantly epithelial differentiation, in comparison, tend to have lower tumor volume and may have more extensive lymph node involvement. Despite the purported predilection for hematoenous spread, lymph node involvement was an important predictor of overall survival in this study.

132 Prevalence of polysomy 17 by OncoScan in Fluorescence In Situ Hybridization (FISH) HER2 Gene amplification by HER2 gene copy number of > 6.0 with HER2/CEP17 ratio of < 2.0 in breast cancer

Sophia Apple[1], *Joyce Murata-Collins*[2]. [1]City of Hope, Duarte, CA, [2]City of Hope

Background: 2013 ASCO-CAP guidelines redefined *HER2* gene amplification to be positive with a *HER2*/CEP17 ratio of $\geq$ 2.0 or *HER2* copy numbers of $\geq$ 6.0 for amplification even if the ratio is < 2.0. We retrospectively evaluated *HER2*/CEP17 ratio <2.0 with the *HER2* copy numbers of $\geq$ 6.0 cases to evaluate both the incidence of this category and whether it is due to polysomy 17.

Design: A total of 1348 cases were included in the study from 1/2014-9/2017. Evaluations of *HER2*/CEP17 ratio, *HER2* copy number, IHC for *HER2* and clinical data were obtained. Polysomy 17 was verified using OncoScan microarrays to determine polysomy of an entire chromosome 17. The OncoScan software focuses on 900 known cancer genes and provides high resolution whole genome screening for tumors with one assay which is superior to reflex RARA test for polysomy.

Results: From 1348 samples, FISH amplified cases with ratio of $\leq$ 2.0 but *HER2* copy numbers of $\geq$ 6.0 were found in 20 (1.48%) cases. Of these cases, only one case had IHC score of 3+, 14 (70%) had 2+, and 5 (25%) had either 0 or 1+. Anti-*HER2* treatment was initiated in 40% of patients. Of eleven specimens positive for *HER2* with an average *HER2* copy number >= 6 signals with *HER2*/CEP17 ratio of <2.0 tested by OncoScan, all specimens were found to be polysomic for chromosome 17 long arm by microarray analysis.

Conclusions: The incidence of *HER2* copy numbers of $\geq$ 6.0 in the setting of *HER2*/CEP17 ratio of <2.0 is rare, 1.4% in our study, all of which had polysomy for chromosome 17 long arm. Our oncologists are not uniformly treating with anti-*HER2* therapy in the setting of *HER2* copy numbers of $\geq$ 6.0 *HER2*/CEP17 ratio of <2.0. OncoScan microarray is a superior test to demonstrate and confirm polysomy of chromosome 17 prior to treatment selection.

133 PD-L1 Protein Expression in Algerian Inflammatory Breast Cancer (IBC) Patients

Hugo Arias-Pulido[1], *Ashley Cimino-Mathews*[2], *Nabila Chaher*[3], *Nancy Joste*[4], *Clifford Qualls*[5], *Max Foisey*[1], *Jonathan Marotti*[6], *Leisha Emens*[7], *Steven Fiering*[1]. [1]Dartmouth Geisel School of Medicine, [2]Johns Hopkins Hospital, Baltimore, MD, [3]Centre Pierre et Marie Curie, [4]University of New Mexico, Albuquerque, NM, [5]The University of New Mexico Clinical and Translational Science Center, Albuquerque, NM, [6]Dartmouth-Hitchcock Medical Center, Lebanon, NH, [7]The Johns Hopkins Hospital

Background: IBC is the deadliest form of locally-advanced breast cancer (LABC), which affects ~2.5% of American breast cancer patients and up to 11% of patients in Northern Africa. Anti-PD-1/PD-L1 therapy for breast cancer is currently being examined in clinical trials, but knowledge of PD-L1 expression in IBC is limited. Here, PD-L1 protein expression in IBC and non-IBC LABC was analyzed by immunohistochemistry (IHC) to determine frequency and association with clinico-pathological characteristics, including response to neoadjuvant therapy.

Design: IHC was performed on tissue microarrays containing 221 IBC and 162 LABC samples obtained from Algerian women using anti-human PD-L1 antibody (clone 5H1). The average percentage of tumor cells and tumor-infiltrating lymphocytes (TILs) exhibiting membrane PD-L1 expression across duplicate tumor cores was calculated; >5% was considered positive. Scoring was blinded to tumor characteristics and patient outcomes. Pathological Complete Response (pCR) was defined as absence of residual invasive or invasive and noninvasive cancer in the breast and lymph nodes following treatment.

Results: LABC showed higher PD-L1 tumor cell expression than IBC (15% vs. 8%; P=0.047). PD-L1 tumor cell positivity correlated with pCR rates in IBC (29% PD-L1+ vs. 7% PD-L1-; P=0.009) and LABC (41% PD-L1+ vs. 16% PD-L1-; P=0.016), and with young age, pre-menopausal status, and nulliparity in LABC (P<0.04 for all). PD-L1 expression in TILs correlated with tumor size, grade, and Ki-67 in IBC (P<0.03 for all); and with pCR rates (26% PD-L1+ vs. 3% PD-L1-; P=0.002) and Ki-67 (P<0.001) in LABC. PD-L1 expression in TILs was associated with disease-free survival in LABC (DFS; P=0.012). Multivariate analysis showed that pCR was the single factor associated with breast cancer-specific survival in both IBC and LABC (P<0.02) and with DFS in LABC (P<0.0001). pCR and PD-L1 expression in TILs were associated with DFS in IBC (P<0.05 for both).

Conclusions: To date, this is the largest evaluation of PD-L1 protein expression in IBC compared to non-IBC LABC patients. PD-L1 expression in TILs was associated with better DFS in IBC, supporting the development of clinical trials testing agents that target the PD-1 pathway in IBC patients.

134 Impact of Oncotype DX Recurrence Score on Adjuvant Chemotherapy Use for Hormone Receptor Positive Breast Cancer and Correlation with Pathologic Features

Simone Arvisais-Anhalt[1], *Amanda Strickland*[2], *Hong Zhu*[3], *Xu Xiaohan*[3], *Venetia Sarode*[4]. [1]UT Southwestern Medical Center, Dallas, TX, [2]UT Southwestern, Dallas, TX, [3]University of Texas Southwestern, [4]University of Texas Southwestern, Dallas, TX

Background: The Oncotype DX (ODX) (Genomic Health, Redwood City, CA) stratifies patients into 3 risk recurrence categories: low (< 18), intermediate (18-30), and high (> 30). The incorporation of the ODX assay in clinical practice has significantly impacted the use of adjuvant chemotherapy (ADCT) in patients with hormone receptor positive breast cancer. Patients with low recurrence score (RS) derive no survival benefit from ADCT while those with high RS have improved survival with ADCT. The management of patients with intermediate RS, however, poses a significant dilemma and clinical decisions regarding the use of ADCT is still being debated. We aimed to examine the relationship between intermediate level ODX RS and clinicopathologic features in search of an association with ADCT use.

Design: We retrospectively analyzed ODX results in 320 patients with early stage hormone receptor positive breast cancer. Patient age, tumor size, histologic type, tumor grade, presence or absence of lymphovascular invasion, ER, PR and Ki67 was correlated with ODX RS. We also determined the association between pathologic variables and ADCT use in patients with intermediate RS.

Results: There were 178 (56%), 122 (38%) and 20 (6%) patients with low, intermediate and high RS, respectively. Overall, 33% of patients received ADCT. 25% of patients with low RS, 36% with intermediate RS, and 95% of patients with high RS were given ADCT. In the 122 patients with intermediate RS, there was no association between pathologic variables and ADCT use, Table 1. However, 60% (12/20) of patients with higher Intermediate RS (26-30) received ADCT versus 31% (32/102) of patients with lower intermediate RS (18-25), p=0.0213.

Intermediate RS	ADCT	No ADCT	p-value
Total = 122	44(36%)	78(63.9%)	
Age	56.2(10.8)	57.7(9.58)	0.23
Tumor Size <=2.0 cm >2.0 cm	1.64(0.9) 34(80.9%) 8(19.0%)	1.67(1.11) 65(83.3%) 13(16.6%)	0.74
Histologic Type: Ductal Lobular Other	 29(67.4%) 6(13.9%) 8(18.6%)	 56(71.7%) 8(10.2%) 14(17.9%)	0.81
Grade: I II III	 8(18.6%) 29(67.4%) 6(13.9%)	 27(34.6%) 45(57.6%) 6(7.6%)	0.14
LVI Present Absent Suspicious	 8(18.1%) 32(72.7%) 4(9.0%)	 10(12.8%) 64(82.0%) 4(5.1%)	0.46
ER%	86.7(19.4)	92.1(14.1)	0.11
PR%	46(36.9)	49.5(34.9)	0.61
Ki67%	22.5(16.2)	21.4(15.7)	0.71

Conclusions: For patients with intermediate ODX RS, the lack of significant association between any of the above clinicopathologic factors and chemotherapy administration raises questions that merit further investigation. Clinical factors such as personal and family history, candidacy to receive chemotherapy, and even patient preference play major roles in driving the decision to receive chemotherapy in these "gray-zone" cases.

135 Basal biomarkers Nestin and INPP4b accurately identify intrinsic breast cancer subtype and predict benefit from anthracycline vs. non-anthracycline based treatment in the CCTG MA.5 phase III randomized clinical trial

Karama Asleh[1], *Dongxia (Doris) Gao*[2], *Dongsheng Tu*[3], *Lois Shepherd*[4], *Mark N Levine*[5], *Kathleen Pritchard*[6], *Torsten Nielsen*[7]. [1]Genetic Pathology Evaluation Center, Vancouver, BC, [2]Jack Bell Research Centre, Vancouver, BC, [3]Queen's University, Kingston, ON, [4]Canadian Cancer Trials Group, Queen's University, Kingston, ON, Canada, [5]3. McMaster University, Hamilton, ON, Canada, [6]Sunnybrook Odette Cancer Center, Toronto, ON, [7]Univ. of British Columbia, Vancouver, BC

Disclosures: Torsten Nielsen: *Royalties*, NanoString Technologies

Background: A growing body of evidence suggests that intrinsic breast cancer subtypes display a different sensitivity to specific chemotherapies. In a prospective-retrospective analysis of the phase III CCTG MA.5 clinical trial randomizing premenopausal women with node-positive breast cancer to adjuvant cyclophosphamide-epirubicin-fluorouracil (CEF) vs. cyclophosphamide-methotrexate-fluorouracil chemotherapy (CMF), patients with HER2-enriched PAM50 subtype were the ones that derived the greatest benefit when treated with CEF vs. CMF. However a trend for a better survival on CMF was shown in the PAM50 basal-like subtype. More accurate immunohistochemical (IHC) biomarkers consisting of nestin positivity or loss of inositol polyphosphate-4-phosphate (INPP4b), have recently been optimized to identify basal-like breast cancers. In this study we examined their capacity to identify intrinsic subtype and predict CEF vs. CMF treatment benefit on the CCTG MA5 clinical trial

Design: 511 Formalin-fixed paraffin embedded blocks of primary tumor from patients in the CCTG MA.5 trial were used to build tissue microarrays. IHC staining and interpretation for nestin and INPP4b followed published methods and was performed by pathologists with no access to molecular data. The performance of nestin and INPP4b panel was assessed against qRT-PCR PAM50 gene expression assay as a standard reference. A prespecified statistical plan was executed independently by the Canadian Clinical Trials Group, testing the primary hypothesis that patients with basal breast cancer – defined as positive for nestin or negative for INPP4b, regardless of ER/PR/HER2 status – would have superior relapse free survival (RFS) on CMF when compared to CEF by interaction test

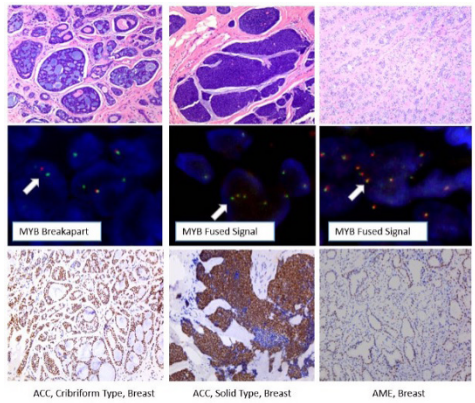
Results: 453 primary tumor samples from CCTG MA.5 were available for IHC biomarker evaluation and clinical outcomes. Positive staining of nestin or loss of INPP4b was observed in 110 (24%) of the total cases and was significantly associated with PAM50 basal-like subtype (p<0.0001). Patients assigned as basals by "nestin+ or INPP4b-" demonstrated a lower benefit from CEF vs. CMF (HR=1.49 [0.72, 3.10]), whereas "nestin- and INPP4b+" displayed a higher benefit from CEF vs. CMF (HR=0.75). The interaction test was significant (p-interaction=0.01)

Conclusions: The nestin/INPP4b IHC panel offers a feasible and inexpensive methodology to identify basal-like patients. IHC basal patients assigned by this panel display a superior RFS when treated with CMF vs. CEF in the adjuvant setting

136 Intraductal Papillomas without Atypia Diagnosed on Core Needle Biopsy: Reasons for Exclusion from a Multi-Center Prospective Trial Following Central Pathology Review

Gabrielle Baker[1], *David Suster*[2], *Tari King*[3], *Faina Nakhlis*[3], *Stuart Schnitt*[4]. [1]Beth Israel Deaconess Medical Center, Boston, MA, [2]Boston, MA, [3]Brigham and Women's Hospital, [4]Brigham and Women's Hospital; Dana Farber Cancer Institute, Boston, MA

Background: Intraductal papillomas (IDP) diagnosed on core needle biopsy (CNB) are frequently managed with surgical excision due to the potential for upgrades to DCIS or invasive carcinoma. Given the wide range in upgrade rates reported in the literature and the retrospective nature of most studies, a multi-center prospective trial is being conducted to better understand the upgrade rate at surgical excision of IDP lacking atypia on CNB. This analysis reports on cases excluded from the study based on central review.



Design: The first 71 patients with a diagnosis of IDP without atypia on CNB enrolled in a multi-center prospective trial (TBCRC-034) represent the population for this analysis. The primary endpoint of this trial is to evaluate the upgrade rate on surgical excision. Selected slides from the CNB and excision specimens for each case were submitted for central pathology review.

Results: Nineteen of 71 cases (27%) were excluded from the study due to a change in diagnosis on central pathology review of the CNB. Of these, 16 (84%) lacked diagnostic features of IDP. Among these 16 cases, lesions that were mistaken for IDP included plicated ducts of variable caliber with associated UDH (n=4); cystic and papillary apocrine metaplasia (n=2); atypical ductal hyperplasia (ADH; n=1); radial scar (n=1); florid UDH (n=1); large irregular ducts (n=1); and fibroadenomatous change (n=1). In 5 cases, there were multiple benign histologic alterations and the lesion misconstrued as IDP was uncertain. Four cases were excluded due to the presence of an atypical ductal proliferation on CNB: 3 had ADH in breast tissue away from the IDP and 1 had ADH in association with the IDP. One case was excluded due to both lack of IDP and presence of ADH. Lobular neoplasia (LN; atypical lobular hyperplasia, classic lobular carcinoma in situ) was present in 3 (4%) of the 71 CNBs; the presence of LN alone did not prompt exclusion since this lesion when incidental is not an indication for excision. Of the 52 IDP remaining eligible for the study, 10 could be categorized as micropapillomas.

Conclusions: The diagnosis of IDP on CNB may be challenging, and in this study the CNB diagnosis was changed in 27% of cases. Overinterpretation of non-IDP proliferations or normal structures as IDP (21%) and an underdiagnosis of ADH by the originating institution (6%) were both observed. To the authors' knowledge, this is the first systematic report of proliferations and normal structures which may be mistakenly diagnosed as IDP in CNB.

137 Pleomorphic Lobular Carcinoma In Situ: Molecular Signature in Comparison to High-Grade Ductal Carcinoma In Situ and Classical Lobular Carcinoma In Situ

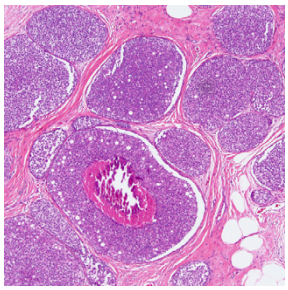
Rania Bakkar[1], *Eric Vail*[2], *Jean Lopategui*[1], *David Engman*[3], *Farnaz Dadmanesh*[2]. [1]West Hollywood, CA, [2]Cedars-Sinai Medical Center, [3]Los Angeles, CA

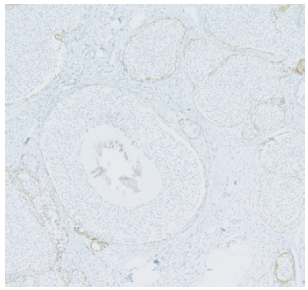
Background: Distinction of pleomorphic lobular carcinoma in situ (PLCIS) from high-grade ductal carcinoma in situ (HGDCIS) is critical because of the differences in the clinical management options of the two entities. Few studies have compared PLCIS, classical LCIS (CLCIS) and HGDCIS without concomitant invasive carcinoma and only one has attempted to assess their comparative molecular profiles. In this study, using next generation sequencing (NGS), we attempted to compare for the first time the molecular profiles of pure PLCIS, HGDCIS and CLCIS.

Design: Twenty-seven cases including PCLIS (n=8), CLCIS (n=8) and HGDCIS (N=11) without concurrent invasive carcinoma were selected via an archival database search. Histology slides along with immunohistochemical studies were independently reviewed by two breast pathologists for accurate categorization. All PLCIS diagnoses were confirmed by absence of E-cadherin expression by immunohistochemistry (see Figures 1&2). Macro-dissection of tissue samples was performed and total nucleic acid was extracted from the tumor tissue. Multiplex PCR amplification using the Ion AmpliSeq Cancer Hotspot Panel v2 followed by emulsion PCR and NGS on the Ion S5 platform was performed.

Results: Five of the 8 PLCIS samples contained a *PIK3CA* mutation (63%) and 3 had a *TP53* mutation (38%). Four of the CLCIS samples (n=8) contained a *PIK3CA* mutation (50%), 2 had a *TP53 mutation* (25%) and 2 had an *ERBB2* mutation (25%). Finally, 3 of the HGDCIS samples (n=11) contained a *PIK3CA* mutation (27%), 3 had a *TP53* mutation (27%), 1 had an *ERBB2* mutation (9%), one had a *PTEN* mutation (9%) and one had a *CDH1 mutation* (9%). All *PIK3CA* and *ERBB2* mutations are previously described activating point mutations and all *TP53, CDH1* and *PTEN* mutations are loss of function mutations (see Table).

	PLCIS (n=8)	CLCIS (n=8)	HGDCIS (n=11)
PIK3CA	5 (63%)	4 (50%)	3 (27%)
TP53	3 (38%)	2 (25%)	3 (27%)
ERBB2	0	2 (25%)	1 (9%)
PTEN	0	0	1 (9%)
CDH1	0	0	1 (9%)





Conclusions: These results are consistent with the known prevalence of *PIK3CA* mutations in both CLCIS (~40-50%) and DCIS (~20-30%). Our data from this pilot study suggest that PLCIS has a molecular signature that is closer to CLCIS than HGDCIS. Analysis of a larger number of samples, perhaps with a larger panel of genes, may allow molecular stratification of these important clinical entities.

138 Digital Breast Tomosynthesis (DBT) increases the detection of invasive lobular carcinoma (ILC); Experience at a tertiary care center

Agnes Balla[1], *Sally Herschorn*[2], *Donald Weaver*[3], *Abiy Ambaye*[4]. [1]University of Vermont Medical Center, Burlington, VT, [2]University of Vermont Medical Center, [3]Univ. of Vermont, Burlington, VT, [4]University of Vermont, South Burlington, VT

Background: Since the FDA approval of DBT in 2011, a large collaborative study found a reduction in the breast screening-recall rate and an increase in invasive carcinoma detection rates when DBT was added to digital mammography.[1] Data on the relative cancer detection rates between subtypes of carcinoma using DBT is lacking. Tomosynthesis (Hologic) was introduced at the University of Vermont Medical Center in 2012 and its use has increased from fewer than 5000 scans in 2012 to over 22,000 examinations per year since 2015. At UVMMC, DBT is offered for screening to all patients and is also used for all diagnostic exams unless the patient has been recalled or followed for calcifications. We report our experience with cancer detection rates after introduction of DBT.

Design: A retrospective analysis of prospectively collected data from UVMMC Breast Cancer Database identified all newly diagnosed invasive and ductal carcinoma in situ (DCIS) between 2008 and 2016 (103 month period). A total of 3,136 patients were included in the study. Invasive carcinomas accounted for 83.2%, and DCIS accounted for 16.8% of the cases. The overall proportion of invasive carcinomas and among invasive carcinomas, the proportions of lobular, ductal and mixed ductal and lobular carcinomas were compared between the pre-DBT and post-DBT periods using Chi-square tests.

Results: 1543 carcinomas were diagnosed in the period between 2008 to June 2012 and 1593 carcinomas were diagnosed after June 2012 after DBT was introduced. Table 1 outlines the proportions of each tumor type.

Tumor type	Pre-DBT n (%)	Post-DBT n (%)	p-value
Total Cases	1543	1593	
Invasive	1244 (80.6)	1364 (85.6)	.0002
ILC	133 (10.7)	190 (13.9)	.01
IDC	1028 (82.6)	1093 (80.1)	.10
IDC/ILC	57 (4.6)	58 (4.3)	.68
Other	26 (2.1)	23 (1.7)	.45
In situ (DCIS)	299 (19.4)	229 (14.4)	.0002

Conclusions: Following introduction of DBT, there was a significant increase in the proportion of invasive carcinoma diagnoses (p=.0002). Among all invasive carcinomas, there was a significant increase in the proportion of ILC diagnoses with DBT (p=.01). These findings support observations made in prior studies that DBT improves the detection of invasive carcinomas in general and ILC in particular, by more clearly depicting architectural distortions and spiculations.[2,3]

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139 Pathologic Features and Clinical Outcomes of HER2 FISH Cases with HER2:CEP17 ratio > 2.0 but < 4 HER2 signals/cell; A Multi-Institutional Study

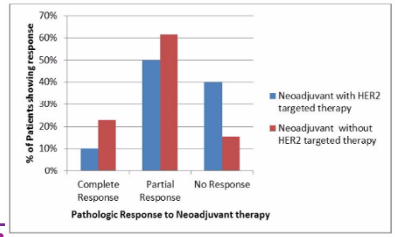
Morgan Ballard[1], *Mirna Toukatly*[2], *Gregory Bean*[3], *Florencia Jalikis*[4], *Gregor Krings*[5], *Rodney Schmidt*[6], *Yunn-Yi Chen*[7], *Suzanne Dintzis*[8], *Megan Troxell*[9], *Robert West*[10], *Richard Sibley*[11], *Kimberly Allison*[10]. [1]Stanford University School of Medicine, Stanford, CA, [2]University of Washington Medical Center, Seattle, WA, [3]UCSF, San Francisco, CA, [4]University of Washington, Seattle, WA, [5]UCSF, San Francisco, CA, [6]Univ. of Washington, Seattle, WA, [7]Univ. of California, San Francisco, San Francisco, CA, [8]Mercer Island, WA, [9]Stanford University Medical Center, Stanford, CA, [10]Stanford University, Stanford, CA, [11]Stanford University Med. Ctr., Stanford, CA

Background: When using dual probe HER2 FISH, breast cancers with low (< 4) mean HER2 signals/cell are currently considered HER2 amplified by ratio if the mean CEP17 control signals are reduced and the HER2:CEP17 ratio is ≥ 2.0 (referred to as Group 2 by 2017 HER2 ISH guidelines). This is a controversial category of HER FISH results with limited clinical data.

Design: The pathology databases from 3 institutions were queried over a 10-year period for breast cancers with HER2 FISH results in the Group 2 category. Additional clinicopathologic features were assessed with specific attention to treatment modalities and response to neoadjuvant therapy.

Results: Of the 8393 breast carcinoma cases with HER2 FISH results, 118 Group 2 cases were identified. The mean age at diagnosis was 56 years and the majority of cases were grade 1-2 (55%) and/or ER positive (78%). Concurrent HER2 IHC was performed on 101 cases, with nearly all cases lacking 3+ protein over-expression (91% of cases 0-2+). Staging was available on 63 patients with 49% Stage I at presentation, 33% Stage II, 13% Stage III and 5% Stage IV.

Clinical follow-up and treatment information was available on 69 (58%) cases, ranging from 2-195 months (median = 56 months). Chemotherapy was given to 82% of patients, 23% also received HER2 targeted therapy. Neoadjuvant chemotherapy was administered in 26 cases (38%), 11 of which included HER2 targeted therapy (chemo(+) HER2) and 13 chemotherapy alone (chemo(-)HER2). Treatment type was not available on 2 cases. None of these cases had 3+ protein over-expression by IHC. Neoadjuvant treatment response specifics were available on 24 cases. Of patients receiving chemo(+)HER2, 36% had no response, 55% had a partial response and 9% had a complete response. Of patients receiving chemo(-)HER2, 15% had no response, 62% had a partial response and 23% had a complete response. (Figure 1) Overall patients with ER negative and low ER expression had better response to therapy. 3 patients receiving neoadjuvant therapy died from disease, 1 received chemo(+)HER2 and 2 chemo(-)HER2.



Conclusions: In this multi-institutional study patients with Group 2 HER2 FISH results frequently received chemotherapy, often without HER2 targeted therapy. When neoadjuvant chemotherapy was given, complete pathologic response was more common in the group that did not receive HER2 targeted therapy. Our results suggest that patients with Group 2 FISH results and 0-2+ IHC receive little benefit from HER2 targeted therapies.

140 Potential Impact of Proposed HER2 FISH Guideline Updates on FISH results; A Multi-Institutional Study

Morgan Ballard[1], *Wendy MacKerricher*[2], *Florencia Jalikis*[3], *Gregor Krings*[4], *Rodney Schmidt*[5], *Yunn-Yi Chen*[6], *Mara Rendi*[7], *Suzanne Dintzis*[8], *Megan Troxell*[9], *Robert West*[10], *Richard Sibley*[11], *Kimberly Allison*[10]. [1]Stanford University School of Medicine, Stanford, CA, [2]Stanford University, Palo Alto, CA, [3]University of Washington, Seattle, WA, [4]UCSF, San Francisco, CA, [5]Univ. of Washington, Seattle, WA, [6]Univ. of California, San Francisco, San Francisco, CA, [7]University of Washington, [8], Mercer Island, WA, [9]Stanford University Medical Center, Stanford, CA, [10]Stanford University, Stanford, CA, [11]Stanford University Med. Ctr., Stanford, CA

Background: In June of 2017 the CAP/ASCO HER2 guidelines committee posted for open comment a proposal for a focused update of the 2013 HER2 Testing Guidelines. This proposal suggests modifying the interpretation criteria of three less common, but problematic HER2 FISH categories; "monosomy like (Group 2)," "co-amplified (Group 3)," and "equivocal (Group 4)." Cases with initial FISH results in these groups would be interpreted in the context of concurrent HER2 IHC results. Cases with 2+ IHC results would be recounted by a second FISH observer. The aim of this study was to evaluate the impact of these proposed changes.

Design: A multi-institution breast cancer HER2 FISH database created from cases over a 10 year period was utilized to determine how interpretations of HER2 would change with the proposed update. Raw HER2 FISH results were interpreted using the 2013 HER2 Guidelines Update and compared to interpretations using the proposed update. Attention was paid to cases changing from positive to negative under the new guidelines, cases requiring concurrent IHC for definitive interpretation and cases needing a FISH recount by a second observer.

Results: A total of 8393 invasive carcinoma cases with HER2 FISH testing were evaluated. Of those cases, 547 were categorized as Groups 2, 3, or 4 based on 2013 criteria, representing 6.5% of all cases (Group 2: 118, Group 3: 72, Group 4: 357). All of these cases would require interpretation in the context of a concurrent IHC test under the proposed update criteria. 455 of these cases had concurrent IHC results (Group 2: 102, Group 3: 66, Group 4: 287). Using the database IHC results and the proposed criteria, HER2 interpretation (without a recount of results) would change from positive to negative for 92% of Group 2, 17% of Group 3 and 94% of Group 4 cases, assuming ISH recounts would not change the result. This would change 4.5% of the total HER2 FISH cases from positive to negative. The cases that changed from positive to negative were 81% ER positive and 58% Grade 1-2. Although recounting FISH was not an option in this study, 61% of cases in Groups 2-4 had 2+ IHC results and would require a blinded recount of the ISH. This represented only 3% of cases in the database. (Table 1).

HER2 FISH Group	Total Cases	Total Cases with IHC	Cases converting from positive to negative	Cases remaining positive (IHC 3+)	Cases requiring ISH recount
Group 2 ("monosomy like")	118	102	94 (92%)	8 (8%)	59 (58%)
Group 3 ("co-amplified")	72	66	11 (17%)	33 (50%)	22 (33%)
Group 4 (equivocal)	357	287	271 (94%)	16 (6%)	196 (68%)

Conclusions: Using a multi-institution HER2 FISH database, the proposed update to HER2 testing results in a small decrease in the number of HER2 positive cases and requires additional work-up for a minority of cases. These cases had otherwise favorable features.

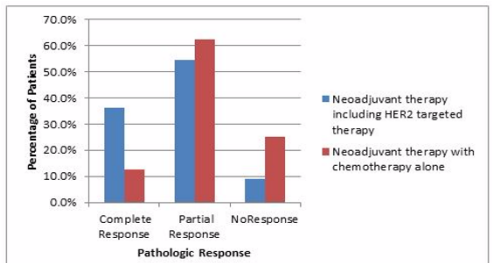
141 Pathologic Features and Clinical Outcomes of Breast Cancers with HER2/CEP17 ratio <2.0 and mean HER2 signals /cell >6.0 by FISH; A Multi-Institutional Study

Morgan Ballard[1], *Mirna Toukatly*[2], *Gregory Bean*[3], *Florencia Jalikis*[4], *Gregor Krings*[5], *Rodney Schmidt*[6], *Yunn-Yi Chen*[7], *Mara Rendi*[8], *Suzanne Dintzis*[9], *Megan Troxell*[10], *Robert West*[11], *Richard Sibley*[12], *Kimberly Allison*[11]. [1]Stanford University School of Medicine, Stanford, CA, [2]University of Washington Medical Center, Seattle, WA, [3]UCSF, San Francisco, CA, [4]University of Washington, Seattle, WA, [5]UCSF, San Francisco, CA, [6]Univ. of Washington, Seattle, WA, [7]Univ. of California, San Francisco, San Francisco, CA, [8]University of Washington, [9], Mercer Island, WA, [10]Stanford University Medical Center, Stanford, CA, [11]Stanford University, Stanford, CA, [12]Stanford University Med. Ctr., Stanford, CA

Background: Breast cancers tested for HER2 gene amplification using dual probe FISH can have synchronously increased HER2 and CEP17 signals, resulting in a negative ratio (< 2.0) but positive result by mean HER2 signals/cell (≥ 6.0) (referred to as "Group 3" of the 5 possible HER2 guidelines ISH result categories). Evidence suggests that this frequently results from "co-amplification" of both regions of chromosome 17. Current HER2 testing guidelines consider these results amplified, and these patients eligible for HER2 targeted treatments. However, evidence is limited regarding clinicopathologic features and clinical outcomes of these cases.

Design: The pathology databases from three institutions were searched for breast carcinomas with HER2 FISH results collected over a ten year period. Results were interpreted using the updated 2013 HER2 FISH testing guidelines. Additional clinical-pathologic features were assessed including, HER2 IHC, ER status, grade, age, stage, treatment modalities including neoadjuvant regimens, and response to neoadjuvant therapy.

Results: Of the 8393 breast carcinoma cases with HER2 FISH results, 72 Group 3 cases were identified. The majority of cases were grade 3 (69%) and/or ER positive (73%). Concurrent HER2 IHC was performed on 65 cases, with 48% of cases being IHC positive, 35% IHC equivocal and 17% IHC negative. Clinical stage at presentation varied with 45% stage I, 23% stage II, 23% stage III, and 9% stage IV. Clinical follow-up and treatment information was available on 55 (76%) of cases, ranging from 3-189 months (median = 53). The rate of metastatic and recurrent disease was high at 53% and 14% respectively, with death occurring in 3 patients. Neoadjuvant therapy was administered in 19 cases, of which 58% received HER2 targeted therapy in the neoadjuvant setting (11 cases). Majority of patients who received HER2 targeted neoadjuvant therapy had either a partial (55%, 6 cases) or complete pathologic response (36%, 4 cases). One patient had no response to therapy. In contrast, the group whose neoadjuvant regimen did not include HER2 targeted treatment experienced less of a response to therapy (5 patients had a partial response and 2 had no response). One patient had a complete pathologic response. (Figure 1)



Conclusions: Although assessment of neoadjuvant chemotherapy response is limited by the small sample size, overall our results support the current classification of co-amplified cases as HER2 amplified with consideration for HER2-targeted treatment.

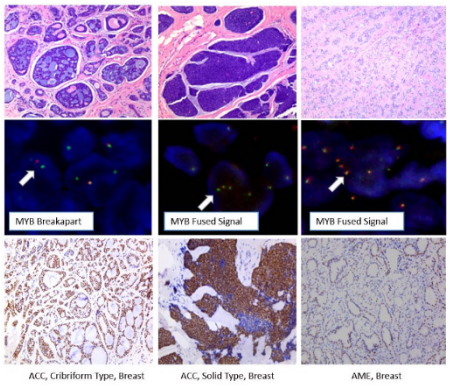
142 MYB Rearrangement and Immunohistochemical (IHC) Expression in Adenomyoepithelioma (AME) of the Breast: A Comparison with Adenoid Cystic Carcinoma (ACC)

Ezra Baraban[1], *Paul J Zhang*[2], *Shabnam Jaffer*[3], *Amanda Lisby*[4], *Michael Feldman*[5], *Ira Bleiweiss*[6], *Anupma Nayak*[7]. [1]Philadelphia, PA, [2]Hospital of the University of Pennsylvania, Media, PA, [3]New York, NY, [4]Hospital of the University of Pennsylvania, [5]University of Pennsylvania, Philadelphia, PA, [6]Hospital of the University of Pennsylvania, Philadelphia, PA, [7]Perelman School of Medicine & Hospital of the University of Pennsylvania, Philadelphia, PA

Background: AME and ACC of the breast have been noted to occur simultaneously, raising the possibility that AME may represent a related or precursor lesion to ACC. ACC of the breast, like its counterpart in the salivary gland, frequently harbors genetic rearrangement of the MYB gene. Recently, expression of MYB by IHC has been demonstrated to be a sensitive and specific marker for ACC, even in the absence of detectable MYB rearrangement. We sought to clarify the relationship between AME and ACC by comparing their rates of MYB expression by IHC and MYB rearrangement by fluorescent in-situ hybridization (FISH).

Design: We retrospectively collected 11 cases of breast-ACC, 11 cases of non-breast ACC (head & neck and lung) and 11 cases of breast-AME from pathology archives. IHC (v-myb+c-myb) as well as FISH for MYB rearrangement using a break-apart probe, were performed on formalin-fixed paraffin embedded sections. IHC (nuclear positivity) was scored for the percentage of positive cells and the staining intensity (1+, 2+, 3+).

Results: Of the 11 breast ACC's, 7 were cribriform type whereas 3 were solid type. Of note, 1 case contained adjacent AME-like areas. Of 11 non-breast ACC's, 8 were cribriform type whereas 3 were solid type. Amongst 11 AMEs, 5 were benign, 2 atypical and 4 demonstrated focal malignant transformation. On FISH analysis, 5 of 11 (45%) breast ACC cases demonstrated MYB gene rearrangement. Of the 6 FISH negative ACC cases, 4 demonstrated MYB expression by IHC, 2 of which were solid type. Overall, 9 of 11 (81%) breast ACC cases demonstrated MYB expression (range, 20-95%). Of the 11 non-breast ACCs, 6 showed MYB rearrangement, all of which were cribriform type. 9 of these 11 cases showed MYB immunoexpression (range, 10-90%), including 3 solid-type cases which were negative by FISH. No MYB rearrangements were detected by FISH in the 11 AME cases. However, 3 of 11 cases (27%) showed weak to moderate MYB expression by IHC (range, 10-40%).



Conclusions: Our results indicate that FISH for MYB gene rearrangement is a specific, but not sensitive marker for ACC of the breast. Immunohistochemistry for MYB may be helpful in highlighting FISH negative cases of ACC, particularly the diagnostically more difficult solid type. None of the AMEs showed MYB rearrangement; however, weak to moderate MYB expression in 27% of AMEs highlights not only a potential diagnostic pitfall, but also shared pathophysiology with ACC worth investigating further at the genomic level.

143 Metaplastic Breast Carcinomas Have an Active Tumor Immune Microenvironment

Amanda Barrett[1], *Janis Taube*[2], *Pedram Argani*[3], *Leisha Emens*[4], *Ashley Cimino-Mathews*[1]. [1]Johns Hopkins Hospital, Baltimore, MD, [2]Baltimore, MD, [3]Johns Hopkins Hospital, Baltimore, MD, [4]The Johns Hopkins Hospital

Background: Metaplastic breast carcinomas are a heterogeneous group of tumors with variable morphology and largely aggressive behavior. The majority are triple negative and currently lack targeted therapy. The tumor immune microenvironment, including the PD-L1 checkpoint axis and the degree of tumor infiltrating lymphocytes (TILs), is a potential area for therapeutic targeting but has not been extensively evaluated in metaplastic carcinomas.

Design: Tissue microarrays (5 replicates of each tumor) containing 13 cases of metaplastic carcinoma were evaluated for the degree of stromal TIL and PD-L1 labeling by immunohistochemistry. The overall TIL density was scored as the percent stromal TILs and subdivided into the following categories: none (0), rare (1; <5% of tumor stromal area), mild (2; 5-10% of tumor stromal area), moderate (3; 11-49% of tumor stromal area), or brisk (4; ≥50% of tumor stromal area). The percentage of membranous PD-L1 labeling in tumor cells was recorded, with <5% considered negative. The percentage of PD-L1 labeling by TILs was scored as none (0), focal (1; ≤5%), moderate (2; 6-49%), or diffuse (3; 50-100%).

Results: Tumors included 6 with spindled morphology, 5 with chondroid metaplasia, 5 with squamoid metaplasia, 1 with osseous metaplasia, and 1 with myxoid morphology (4 tumors displayed more than one morphology). All tumors were triple negative (in one case HER2 was not tested). The majority of tumors (62%) contained rare TILs; 38% contained mild-moderate TILs, and no case contained >50% stromal TIL. The majority (62%) of tumors expressed PD-L1, and the majority (77%) contained moderate PD-L1+ TIL. No tumor contained diffuse PD-L1+ TIL. PD-L1+ carcinomas were associated with older average age at presentation compared to PD-L1- carcinomas (72 years vs. 53 years, p = 0.02), and PD-L1+ carcinomas had greater PD-L1+ TIL in the tumor bed than PD-L1- carcinomas (100% moderate PD-L1+ TIL vs. 40%, p = 0.04). PD-L1+ carcinomas had a greater degree of TIL (63% mild-moderate TIL) compared to PD-L1- carcinomas (100% rare TIL), although this did not reach statistical significance (p=0.08). There was no association between metaplastic morphology and PD-L1 labeling or degree of TIL in this data set.

Conclusions: Metaplastic carcinomas contain an active tumor immune microenvironment, evidenced by the presence of PD-L1+ carcinoma cells and TIL within the tumor. The findings in this study support further exploration of the tumor microenvironment and a potential role for immunotherapy.

144 Malignant Phyllodes Tumors Express PD-L1 Independent of Degree of Tumor Infiltrating Lymphocytes

Amanda Barrett[1], *Janis Taube*[2], *Pedram Argani*[3], *Leisha Emens*[4], *Ashley Cimino-Mathews*[1]. [1]Johns Hopkins Hospital, Baltimore, MD, [2]Baltimore, MD, [3]Johns Hopkins Hospital, Baltimore, MD, [4]The Johns Hopkins Hospital

Background: Breast phyllodes tumors (PTs) are rare fibroepithelial lesions with variable risk for local recurrence and metastasis, especially malignant PTs. The standard management of malignant PTs is complete excision, with limited utility of neoadjuvant therapies. A recent study of malignant PTs reported PD-L1 labeling in a subset of tumors [Oncotarget 2016;7:1707-1716], suggesting that immune checkpoint inhibition and the tumor microenvironment could be further explored across the spectrum of fibroepithelial lesions of the breast.

Design: Tissue microarrays (TMAs) containing 10 fibroadenomas, 10 benign PTs, 10 borderline PTs, and 15 malignant PTs were evaluated for the degree of TIL and PD-L1 labeling by immunohistochemistry. The PTs were sampled with 5 cores per tumor, and the fibroadenomas were sampled with 2 cores per tumor. The overall TIL density within the stromal component of the tumor was subdivided into the following categories: none (0), rare (<5% of tumor area), moderate (5-49% of tumor area) or brisk (≥50% of tumor stromal area). The percent of membranous PD-L1 labeling in neoplastic fibroepithelial stromal tumor cells was recorded, with <5% considered negative. The percent of PD-L1 labeling by TILs was scored as none (0), focal (1; ≤5%), moderate (2; 6-49%), or diffuse (3; 50-100%).

Results: All tumors contained only rare (<5%) TIL, and the majority of TIL (60-80% across tumor types) displayed PD-L1 labeling. PD-L1 labeled the neoplastic cells in 80% (n=12/15) of malignant PTs, compared to 10% (n=2/20) benign/borderline PTs and 0% fibroadenomas (p=0.0001). The percentage of PD-L1 labeling in the malignant PT neoplastic cells ranged from 5-30% of cells in the TMA core. There was no association between PD-L1 labeling in the neoplastic cells and the degree of TIL or the degree of PD-L1 TIL positivity in any fibroepithelial tumor type. Metastases occurred in 25% of PD-L1+ malignant PTs in comparison to 0% of PD-L1- malignant PTs (significance limited by sample size).

Conclusions: TILs are rare across fibroepithelial tumor subtypes, yet the majority of malignant PTs display stromal PD-L1 labeling compared to other tumor types. This degree of PD-L1 labeling in the absence of significant inflammation suggests constitutive PD-L1 expression in malignant PTs, in contrast to the adaptive PD-L1 expression seen with brisk immune infiltrates, and supports further study of the mechanisms of PD-L1 overexpression in malignant PTs and consideration for PD-1/PD-L1 inhibition.

145 Molecular Characterization of Small Cell/ Neuroendocrine Carcinomas of the Breast

Gregory Bean[1], *Sandra J Shin*[2], *Elizabeth Hosfield*[6], *Kirk Jones*[4], *Yunn-Yi Chen*[4], *Gregor Krings*[5]. [1]UCSF, San Francisco, CA, [2]Albany Medical College, Albany, NY, [3]Kaiser Permanente, Oakland, CA, [4]Univ. of California, San Francisco, San Francisco, CA, [5]UCSF, San Francisco, CA

Background: The WHO classifies neuroendocrine carcinomas (NEC) of the breast into three categories: 1) well-differentiated neuroendocrine (NE) tumors, 2) invasive ductal carcinomas (IDC) with NE differentiation, and 3) poorly-differentiated NEC/small cell carcinomas (SCC). Classification of high grade (HG) NEC that do not clearly meet SCC criteria is not well defined, and distinction from SCC can be challenging in practice. In lung, SCC show universal inactivation of *TP53* and *RB1*, but little is known about the genetics of SCC and other HG NEC in the breast. We profiled 6 SCC and 7 HG NEC not meeting SCC criteria by capture-based next generation sequencing (NGS) to determine whether these tumors are genetically related.

Design: Thirteen NEC of the breast were studied. All cases were HG and positive for at least 1 NE marker by immunohistochemistry; 6 showed classic small cell morphology and 7 showed large cell, intermediate cell or mixed features. DNA was extracted from tumor and matched normal tissue for NGS. DCIS in 2 cases (1 SCC, 1 HG NEC) was separately sequenced; 1 HG NEC also included separately sequenced conventional IDC. NGS targeted coding regions of 480 cancer genes. Single nucleotide variants, insertions/deletions and copy number alterations (CNA) were evaluated.

Results: *TP53* and *RB1* co-alterations were identified in 6/6 (100%) SCC and 1 HG NEC with mixed small and large cell features; no (0/6) HG NEC without SCC features had *TP53*/*RB1* co-alteration. Other recurrent aberrations included *PTEN* inactivation in 3/6 (50%) SCC, hotspot *PIK3CA* mutations in 3/13 NEC (1/6 SCC, 2/7 HG NEC), amplification of *ZNF703*/*GPR124* in 5/13 NEC (2/6 SCC, 3/7 HG NEC), and *FGFR1*

amplification in 2/13 NEC (1 SCC, 1 HG NEC). DCIS and invasive NEC showed identical pathogenic mutations with additional CNA in invasive tumor. Conventional IDC in a mixed (IDC-HG NEC) showed identical pathogenic mutations, with additional CNA in the NEC.

Case #	Cytology/Morphology	ER/PR/HER2	TP53 aberration	RB1 aberration
small cell carcinomas				
SCC1	small	-/-/-	+	+
SCC2	small	NA	+	+
SCC3	small	-/-/-	+	+
SCC4	small	+/+/-	+	+
SCC5	small	+/+/-	+	+
SCC6	small	-/-/-	+	+
high grade neuroendocrine carcinomas				
HG NEC1	mixed small & large	-/-/-	+	+
HG NEC2	intermediate	+/+/eq	-	-
HG NEC3	large	-/-/-	+	-
HG NEC4	large	-/-/-	+	-
HG NEC5	intermediate to large	-/-/-	-	-
HG NEC6	intermediate to large	+/+/-	-	-
HG NEC7	intermediate to large	+/+/eq	-	-

NA=not available; eq=equivocal by IHC and FISH

Conclusions: SCC of the breast shows co-alteration of *TP53* and *RB1*, similar to lung SCC. HG NEC not meeting morphologic SCC criteria lack this genetic feature and are genomically more heterogeneous. If validated in a larger cohort, *TP53*/*RB1* testing may be diagnostically useful in challenging cases. The NE component of mixed IDC-HG NEC may evolve from conventional IDC.

146 The clinicopathologic and genomic features of fibrotic foci in breast cancer - results from the TCGA cohort

Francisco Beca[1], *Julia Tsang*[2], *Kristin C Jensen*[3], *Kimberly Allison*[4], *Gary Tse*[5]. [1]Stanford University School of Medicine, Stanford, CA, [2]Prince of Wales Hosp., [3]Stanford Hospital, Stanford, CA, [4]Stanford University, Stanford, CA, [5]Prince of Wales Hosp, Shatin, NT, Hong Kong

Background: Although not routinely reported, fibrotic foci (FF) has been forwarded as a morphologic feature of invasive breast cancer (IBC) with both prognostic and predictive significance. It is one of the criteria of our reported morphologic-molecular recurrence predictive (MMRP) model (Ni YB et al., 2014). In this study we aimed at investigating the clinicopathologic and molecular features with the presence of FF in IBC to further understand its pathologic relevance.

Design: A large cohort of IBC (N=1167) was examined for presence of FF and correlated with clinico-pathological features, biomarker expression and patient survival. Further in-depth analyses using The Cancer Genome Atlas (TCGA) cohort included molecular features as PAM50 subtypes, DNA methylation subtypes (Illumina Infinium DNA chips), microRNA subtypes (Illumina sequencing) and reverse-phase protein assay (RPPA) subtypes (MD Anderson RPPA Core Facility).

Results: FF was seen in 23.5% (274/1167) of the current cohort. It was associated with increased tumor grade (p=0.002), higher pT (p=0.040), higher pN (p=0.012) and increased patients' age (p=0.006). FF was found to be an independent factor for OS (HR=1.771, p=0.006) and DFS (HR=1.816, p=0.001) when adjusted for age, grade, lymphovascular invasion, pT, pN and IHC surrogate molecular subtypes. Among different IHC surrogate subtypes, FF associated with poor survival in particularly luminal B and basal like subtypes. Of the 850 annotated cases for FF from the TCGA cohort (Heng YJ et al., 2017), FF was seen in 31.2% (n=265). Similarly, FF was observed to be associated with decreased DFS in PAM50 basal breast cancer patients (p=0.04) and decreased OS in patients with PAM50 luminal, stage II breast cancer (p=0.0034). Interestingly an independent association with worse survival in patients with Stage II IBC was also observed (DFS p=0.005 and OS p=0.018). Molecularly from TCGA data, FF was associated with the RPPA subtypes (p=0.011), CN clusters (p=0.028) and PAM50 proliferation score (p<0.001).

Conclusions: The presence of FF in breast cancer has important clinical, pathologic and molecular associations. These effects could be dependent on breast cancer subtype and stage. Continued research in this topic is warranted to elucidate if FF can be used as a morphologic feature surrogate of biologic processes associated with tumor aggressiveness.

147 Prognostic significance of PD-L1 expression on cancer, stromal, or immune cells in triple-negative breast cancer

Carolyn E Behrendt[1], *Daniel B Schmolze*[2], *Peter P Lee*[3], *Sophia Apple*[4]. [1]Beckman Research Institute of the City of Hope, Duarte, CA, [2]City of Hope National Medical Center, Duarte, CA, [3]Beckman Research Institute of the City of Hope, [4]City of Hope, Duarte, CA

Background: Expression of programmed death ligand 1 (PD-L1) in human breast tumors is associated with lymph node positivity, higher histologic grade, hormonal receptor-negative status, and shorter overall survival. The prognostic value of PD-L1 is less clear in triple-negative breast cancer (TNBC). Our research questions were: In primary TNBC, is tumor expression of PD-L1 prognostic of recurrence-free survival (RFS)? If so, is PD-L1 expression in stroma or immune cells as prognostic as PD-L1 in cancer cells?

Design: Women with newly-diagnosed, primary TNBC who underwent surgery during 2002-2010 without prior neoadjuvant treatment were eligible for study. Tissue microarray was constructed using 1-6 cores of invasive ductal carcinoma (IDC) per patient. The cores underwent immunohistochemical staining for PD-L1 (FDA-approved Kit, clone 22C3) and were analyzed for expression on 3 types of cells: tumor; stroma; and mixed immune cells (lymphocytes, macrophages, histiocytes). Two board-certified pathologists working independently scored each core for percentage positive for PD-L1 among each of the 3 cell types. Each pair of scores was averaged, and the resulting PD-L1 percentage positive was categorized as Low (0-5%), Intermediate (>5%-10%), or High (>10%). The primary risk factor, High PD-L1 in tumor, and secondary risk factors, High PD-L1 in stroma or immune cells, were investigated for association with RFS using proportional hazards regression applied to IDC cores rather than patients.

Results: Subjects (n=100, age 55.8±12.5 years at TNBC diagnosis) contributed 281 cores of IDC. Distribution of Low, Intermediate, and High PD-L1 differed between cancer, stroma, and immune cells (p<0.0001). During follow-up (median 3.5 years, range 3 days–9.4 years), 26 subjects experienced recurrence, and another 5 died without recorded recurrence. Nine subjects had at least one core of IDC with High PD-L1 among cancer cells; these 9 tumors were Grade 3, poorly differentiated, and basal-like in morphology. High PD-L1 in cancer cells was associated with reduced risk of recurrence or death relative to Low PD-L1: Hazards Ratio 0.44 (95% Confidence Interval 0.19, 0.97), independently of age, distant metastasis, tumor size and invasiveness. Intermediate PD-L1 in cancer cells was not associated with RFS. In addition, High PD-L1 in stroma or in immune cells was not associated with RFS.

Conclusions: In our TNBC series, PD-L1 expression by more than 10% of cancer cells per core of IDC is independently associated with better recurrence

148 Breast cancers with Magee Equation score of less than 25 and mitosis score of 1 do not require Oncotype DX® testing: A Value Study.

Rohit Bhargava[1], *Beth Clark*[2], *David Dabbs*[1]. [1]Magee-Womens Hospital of UPMC, Pittsburgh, PA, [2]University of Pittsburgh, Pittsburgh, PA

Background: Magee Equations (MEs) were derived from routine histopathologic and immunohistochemical (IHC) data to estimate onco*type* DX® (ODX) recurrence score (Flanagan M et al, 2008. PMID: 18360352 and Klein ME et al, 2013. PMID: 23503643). Recently, ME3 has also been validated in the neoadjuvant setting as a stand-alone test to predict for pathologic complete response to chemotherapy (Farrugia et al, 2017. PMID: 28548119). In this value study, we hypothesized that cases with ME scores of less than 25 and mitotic activity score of 1 show actual ODX score of less than 25. In such cases, ODX testing will lack value as these patients are typically not offered chemotherapy.

Design: Clinical requests for ODX testing were prospectively reviewed. Patient and tumor characteristics were recorded for each case from pathology reports for quality assurance purposes. Additionally, tumor slide was also reviewed by the study experts for tumor grading. ME scores were calculated from the data in the pathology report as well as based on expert review. If all 3 equation scores were in low risk (less than 18) or in high risk (31 or higher) category, the cases were labeled as "do not send". If 1 or all 3 equation scores were in intermediate risk category (and all scores less than 25), cases were again labeled as "do not send" if mitotic activity score was 1.

Results: Total of 118 cases have been accrued to date in this value/quality assurance project with available ODX scores. Of these 118 cases, 80 (68%) were predicted (based on original report) to be low/intermediate risk with estimated ODX score less than 25 or clearly high risk with the decision "DO NOT SEND". ODX testing was correctly declined in 76 of 80 cases, i.e. in 95% cases (table 1).

Expert review additionally classified 7 more cases in the "do not send (estimated score less than 25)" category, increasing the correctly declined case number to 83 out of 87 or in 95.4% cases (table 2).

Table 1:

Prediction based on Magee Equations and mitosis score derived from original reports	ODX recurrence score less than 25	ODX recurrence score 25 or higher	Total
Do NOT send (Low / intermediate risk-estimated score less than 25)	75	3*	78
Do NOT send (Clearly high risk)	0	2**	2
Total	75	5	80

*Actual ODX of 25, 26 and 33; **Actual ODX of 28 and 57.

Table 2:

Prediction based on Magee Equations and mitosis score derived from expert review	ODX recurrence score less than 25	ODX recurrence score 25 or higher	Total
Do NOT send (Low / intermediate risk-estimated score less than 25)	82	3*	85
Do NOT send (Clearly high risk)	0	2**	2
Total	82	5	87

*Actual ODX of 25, 26 and 33; **Actual ODX of 28 and 57.

Conclusions: (1) Breast cancer cases with Magee Equation scores of less than 25 and mitosis score of 1 do not require ODX testing as ODX testing lacks clinical value in this setting.

(2) Magee Equations should be utilized in routine clinical practice to determine cases that will not benefit from ODX testing.

(3) Based on these results, 65-75% of all clinically requested ODX tests lack clinical value and are not necessary.

149 Prediction of Breast Cancer Growth Rate In Vivo and Its Clinical Implications

Shristi Bhattarai[1], *Sergey Klimov*[2], *Anthony Wormall*[3], *Remus M Osan*[4], *Helen C Burrell*[5], *Ian Ellis*[6], *Padmashree Rida*, *Ritu Aneja*, *Emad Rakha*[7]. [1]Georgia State University, Atlanta, GA, [2]Roswell, GA, [3]Division of Cancer and Stem Cells, School of Medicine, University of Nottingham and Nottingham University Hospitals NHS Trust, City Hospital Campus, Nottingham, United Kingdom, [4]Georgia State University, [5]Nottingham University Hospitals NHS Trust, Nottingham, [6]City Hospital, NHS Trust, Nottingham, England, [7]University of Nottingham, Nottingham, England

Background: Breast tumors have highly variable rates of growth. *In vivo*, the breast cancer growth rate is governed by a number of inter-related clinicopathological and biological parameters and is carefully regulated. This study aimed to assess the molecular and clinicopathological determinants of breast cancer (BC) growth rate *in vivo*.

Design: The study group comprised female BC patients who participated in and were diagnosed through a screening program. For these patients, a pair of serial mammograms were available wherein the second screening mammogram shows a clearly visible tumor and on retrospective review, it was found that a small tumor was missed in the previous screen (n = 114). Tumor volume, at both time points, was estimated with mammography measurements. These estimated volumes, along with the span between measurements, were fit into a growth rate index. Patients were thresholded into fast and slow growth groups based on this *in-vivo* growth rate and, to determine the prognostic relevance, analyzed with survival analysis. A machine learning algorithm was used to determine if various combinations of biomarkers, obtained at diagnosis, could act as a surrogate for this growth rate. After cross-validating the accuracy of this algorithm, a large independent validation set of 1241 BC patients was used to confirm the clinical value of this metric.

Results: We found that, 10-year breast cancer-specific survival (BCSS) of patients stratified into the slow (BCSS = 92%) and fast (BCSS = 71%) *in-vivo* growth rate was significant (p = 0.03) after controlling for grade, size, and NPI (p = 0.03). Using Ki-67, the mitotic Index, and tumor size we were able to train a KNN classifier which, after 5-fold cross-validation, was able to classify patients into the growth rate groups with >70% accuracy. Using these markers on the naïve dataset resulted in very significant survival difference (p <0.001) between patients with fast (BCSS = 72.91%) and slow (BCSS = 92.28) growing tumors. The model retained significance (P = 0.03) when controlling for NPI, grade, and molecular subtype.

Conclusions: Assessment of primary tumor histologic grade, mitotic counts, and Ki67 expression can be used as a predictor of in vivo tumor growth rate. Overall, the study proposes that growth rate should be taken into consideration in addition to hormone receptor status when sub-typing breast carcinomas, and reveals prognostic information and evidence-based data with relevance to medicolegal practice.

150 Dachsous2 Expression is Associated with Adverse Tumor Characteristics in Axillary Node Negative Breast Cancer

Alina Bocicariu[1], *Dushanthi Pinnaduwage*[2], *Hongtao Zhang*[3], *Helen McNeill*[4], *Anna Marie Mulligan*[5], *Irene Andrulis*[4]. [1]University of Toronto, University Health Network, Toronto, ON, [2]Sinai Health System, [3]Lunenfeld-Tannenbaum Research Institute, Toronto, ON, [4]Lunenfeld-Tannenbaum Research Institute, Toronto, [5]University of Toronto- University Health Network

Background: Dachsous2 (Dchs2) is a cell-adhesion molecule involved in planar cell polarity (PCP) and Hippo pathway dependent tissue growth. While the role of Dchs2 in breast cancer (BC) has not been studied, misregulation of the Hippo pathway is reported. Using gene expression profiling on tumors from women with axillary lymph node negative (ANN) BC, we found Dchs2 to be one of the most highly discriminatory genes in identifying women with early relapse (p=1.1 x 10-5, SAM and PAM moderated t-test; unpublished data). To further investigate the role of Dchs2 in BC progression, we examined its immunohistochemical (IHC) expression in tumors from a large multicentre prospectively accrued series of women with ANN BC.

Design: Tissue microarrays (TMAs) were constructed from tumor blocks of 887 patients in the original ANN cohort. These were stained with a Dchs2 antibody and tumoral expression was scored using the Allred method. Hormone receptors, HER2, Ki-67, CK5, EGFR, p53, and T-bet status were determined using IHC and/or biochemical methods. Tumors were assigned to luminal A, luminal B, HER2, and basal subtypes based on the expression of IHC markers. Chi-square test was used to analyze Dchs2 associations with clinicopathologic variables, IHC markers, and molecular subtype. Analysis of Dchs2 associations with disease-free and overall survival in breast cancer subtypes is ongoing.

Results: Dchs2 positive tumor status was associated with larger tumor size (p=0.0129), higher histologic grade (p<0.0001), ER and PR negativity (p<0.0001), CK5 positivity (p<0.0001), EGFR positivity (<0.0001), high Ki67 (p<0.0001), p53 positivity (p<0.0001) and a high T-bet positive lymphoid cell infiltrate (<0.0001). When stratified according to molecular subgroup, Dchs2 positive tumor status was significantly associated with the basal subgroup (p<0.0001) with 82% of Dchs2 positive tumors being assigned to this group.

Conclusions: Dchs2 expression is associated with adverse prognostic parameters including the basal subgroup in the largely good prognostic group of ANN BC. This observed association between Dchs2 expression and aggressive tumor characteristics may indicate a functional role for the protein in BC development and progression. A greater understanding of this role will provide insights into BC progression, particularly in the basal group, and may provide opportunities for new therapeutic strategies.

151 Clinical Significance of the AJCC 8th Edition Prognostic Staging System for Triple Negative Breast Cancer (TNBC)

Ian Brain[1], *Amy Gillgrass*, *Mark N Levine*[2], *Greg Pond*[3], *Phillip Williams*[4], *Anita Bane*[5]. [1]McMaster University, Hamilton, ON, [2]McMaster University, Hamilton, ON, Canada, [3]McMaster University, [4]Juravinski Cancer Center, Hamilton, ON, [5]Hamilton, ON

Background: Breast cancer is staged using an anatomic staging system based on tumour size (T), nodal status (N) and presence or absence of systemic metastases (M). The new AJCC 8th Edition Cancer Staging Manual has introduced a prognostic stage group for breast cancer that incorporates both the traditional TNM parameters together with tumour grade, estrogen receptor (ER,) progesterone receptor (PR) and HER2 status.'. In this study, we examined the clinical significance of the prognostic stage group in a well annotated cohort of TNBCs with a minimum of 5 years clinical outcome data and compared it to the anatomic staging system for the same cohort of patients.

Design: 189 TN primary invasive breast cancers diagnosed and treated between 2004-2011 were assembled from the pathology archive of Hamilton Health Sciences. Clinical and tumour characteristics were collated, together with treatment received and patient outcome. Kaplan-Meier methods were used to estimate survival at 5 years. Log-rank test was used to compare survival outcomes between patients in

different stages.

Results: Of the 189 patients, 170 (89.9%) were classified into a prognostic stage that was different to the anatomic stage. Of note with the prognostic staging system there were no TNBCs that were prognostic stage I, as all anatomic stage I tumours became prognosis stage II, and all anatomic stage II patients became prognosis stage III. The 5-year survival for anatomic stage I, II and III patients was 96.1% (95% CI 85.3, 99.0), 84.2% (95% CI 76.1, 89.8) and 25.3% (95% CI 67.8, 82.4) respectively. Whereas, the 5-year survival for prognosis stage II and III patients was 96 % (95% CI 85.3, 99.0) and 76% (95% CI 67.8, 82.4) respectively (Table 1). Furthermore, the 5-year survival for the 117 anatomic stage II patients who were upstaged to prognosis stage III was significantly better (84.2%, 95% CI 76.1, 89.8)) than the 19 anatomic stage III patients who remained at stage III on the prognosis staging system (25.3%, 95% CI 8.6, 46.2)

Table 1. TNBC Survival by Anatomic and Prognosis Stage

Marker	Type	N	N (%) Deaths	5-year (95% CI)	p-value
Anatomic Stage	I	53	5 (9.4)	96.1 (85.3, 99.0)	<0.001
	II	117	21 (17.9)	84.2 (76.1, 89.8)	
		19	14 (73.7)	25.3 (8.6, 46.2)	
Prognosis Stage	II	53	5 (9.4)	96.1 (85.3, 99.0)	0.011
	III	136	35 (25.7)	76.0 (67.8, 82.4)	

Conclusions: The prognostic stage for TNBCs has worse discrimination in predicting patient outcome than the anatomic stage. The decreased prognostic ability of the 'prognosis stage' appears to be due to reduced variability and hence reduced discriminatory power.

152 Impact of Oncotype DX Recurrence Score on Breast Cancer Prognostic Stage Group

Andrea C Breaux[1], *Mary Ann Sanders*[2], *Bradley M. Turner*[3], *Xiaoyong Wu*[4], *Shesh N Rai*[5], *Elizabeth C Riley*[5]. [1]University of Louisville, Louisville, KY, [2]Louisville, KY, [3]University of Rochester Medical Center, Rochester, NY, [4]University of Louisville, Louisville, KY, [5]University of Louisville

Background: The 8th edition of the AJCC Staging System for breast cancer (BC) was published in October 2016 with implementation scheduled for January 2018. The most significant change was the creation of the prognostic stage group (PSG) which is determined by using a 4 page table consisting of 152 categories of different combinations of anatomic stage group (ASG), histologic grade (GR), estrogen receptor (ER), progesterone receptor (PR), HER2, and Oncotype DX Recurrence Score (RS) of 0 to 10, yielding a PSG that is either the same, better or worse than ASG. Tumor size and lymph node status used to determine ASG, as well as GR, ER, PR, and HER2 are required components of synoptic reporting. In contrast, Oncotype DX, a clinically validated 21 gene RT-PCR assay that predicts chemotherapy benefit in patients with ER+/HER2- invasive BC, is not routinely performed for all BC and incurs a costly fee. Implementation of PSG may result in an increase in ordering Oncotype DX without a better understanding of its impact on staging. The purpose of this study is to determine the proportion of cases that saw a change in PSG as a result of Oncotype DX RS, and to characterize the pathologic features of these cases.

Design: This is a retrospective review of pathology reports from cases of ER+/HER2- invasive BC with Oncotype DX RS results from 2006 to 2017 at two academic institutions. Clinicopathologic data recorded include GR, ER, PR, HER2, T stage, N stage, Ki-67 and Oncotype DX RS. ASG, PSG, and PSG without Oncotype DX RS were determined for each case and compared. An appropriate statistical test, either the X^2 test or t-test, was used and $P<.05$ was considered significant.

Results: A total of 808 cases were identified. Of these, ASG and PSG were the same in 389 cases (48%), PSG was better than ASG in 249 cases (31%), PSG was worse than ASG in 105 cases (13%), and no PSG was identified for 65 cases (8%). Distribution of stage groups are provided in Table 1. Oncotype DX RS of 0 to 10 occurred in 166 cases (21%). Of these, PSG stayed the same in 112 cases (67%) and was downstaged in 54 cases (33%). Thus, Oncotype DX RS affected staging in 7% (54/808) of cases. Changes in PSG due to low Oncotype DX RS were significantly associated with T stage, N stage, GR, and Ki-67 (Table 2).

Table 1: Distribution of ASG and PSG (n=808)

Stage	ASG	PSG	PSG without Oncotype DX RS
IA	431	407	352
IB	19	253	303
IIA	265	55	58
IIB	81	9	10
IIIA	11	14	14
IIIB	0	4	4
IIIC	1	1	1
No PSG	--	65	66

Table 2: Impact of low Oncotype DX RS on PSG and characteristics with significant association

Characteristics	PSG, no change (112 cases) n (%)	PSG downstaged (54 cases) n (%)	P value
T Stage (n=166)			< .001
1	93 (83%)	7 (13%)	
2	14 (13%)	47 (87%)	
3	5 (4%)	0 (0.0)	
N Stage (n= 166)			< .001
0	78 (70%)	54 (100%)	
1	34 (30%)	0 (0%)	
Grade (n= 166)			< .001
1	60 (54%)	23 (42%)	
2	52 (46%)	23 (43%)	
3	0 (0%)	8 (15%)	
Ki-67 (n= 153)			0.003
< 20%	89 (86%)	33 (66%)	
≥ 20%	14 (14%)	17 (34%)	
Unavailable	9	4	

Conclusions: Our data shows that Oncotype DX RS impacts PSG in a minority of cases, however, our data also shows Oncotype DX RS is most beneficial for pT2N0 tumors that are GR1-2, ER+/PR+/HER2- with low Ki-67 by downstaging PSG.

153 Filling the TAILORx Gap: Survival Benefit from Chemotherapy Using Data from the NCDB

Jane Brock[1], *Susan Lester*[1], *J. Bryan Iorgulescu*[2]. [1]Brigham & Women's Hospital, Boston, MA, [2]Brigham and Women's Hospital, Boston, MA

Background: Chemotherapy is not offered for survival benefits of ≤1%. Beyond this threshold, oncologists discuss with patients their preference and make chemotherapy decisions accordingly. The OncotypeDX Recurrence Score (RS) (Genomic Health) is used by oncologists to identify patients who benefit from the addition of chemotherapy to endocrine therapy. In the TAILORx trial those randomized to receive chemotherapy with RS 11-25 have had too few events to trigger analysis, suggesting there is no chemotherapy-related survival benefit for any RS of ≤25 in the study population at ~10 years of follow up. The current lack of data with mid-range RS (11-31) creates difficulties in the decision-making process regarding benefit from chemotherapy. We have examined differences in overall survival (OS) and hazard ratio of death (HR) using data from the National Cancer Database (NCDB) to fill the gap.

Design: The NCDB includes ~70% of newly diagnosed cancers in the US and was queried for pT1c-T2, N0, M0, grade 2 (G2) and 3 (G3), ER+/HER2- invasive breast carcinomas from 2010-14. 5yr OS was estimated by Kaplan-Meier and compared by log-rank test.

Results: Of 95,845 patients with T1c/T2 G2/G3 tumors, RS was obtained on 55.2% of T1c and 51% of T2 tumors. 5yr OS and HR of death with and without chemotherapy was calculated for RS groups 11-25, 26-28, and 29-31. There was a 5yr OS benefit from receiving chemotherapy in all RS groups, including 11-25 (98.1% vs 96.2%, p<0.001). In chemotherapy treated patients, the OS benefit for RS 11-25 was 1.9% (p<0.001), for RS 26-28 was 4.6% (p=0.03), and for RS 29-31 was 8.9% (p=0.008). pT influenced HR of death adjusted for grade and treatment and unadjusted 5yr OS benefit from addition of chemotherapy in RS groups (see table). Small event numbers likely precluded significance in 2 subgroups.

	RS	n	% Chemo	HR	p-val	% 5yr OS Benefit	p-val
T1c	11-25	18,313	17%	0.35	<0.001	1.7	<0.001
	26-28	1,599	63%	0.42	0.05	5.8	0.03
	29-31	1,098	76%	0.79	0.64	2.2	0.27
T2	11-25	8,178	24%	0.51	0.005	2.7	0.01
	26-28	799	64%	0.62	0.26	2.8	0.23
	29-31	612	79%	0.36	0.02	23.7	0.004

Conclusions: Although follow up is short (<5yrs) and treatments not randomized, the data reflects clinical practice and clearly highlights survival benefits from chemotherapy in mid-range RS. It provides insight for how pT alters OS, complicating RS based chemotherapy decisions. Benefits are significant but small in the 11-25 range (1.7% T1c and 2.7% T2), but reach 5.8% for RS 26-28 in T1c, and higher for RS 29-31 (8.9%) for T1c/T2.

154 The Pathological Response of TNBC to Neoadjuvant Chemotherapy can be Predicted by PD-L1 Expression

Bruna Cerbelli[1], *Angelina Pernazza*[2], *Andrea Botticelli*[2], *Domenico Campagna*[3], *Giulia d'Amati*[4], *Leopoldo Costarelli*[5]. [1]Sapienza, University of Rome, Rome, Italy, [2]Sapienza, University of Rome, [3]San Giovanni-Addolorata Hospital, Rome, Italy, [4]Sapienza University of Rome, Roma, Italy, [5]San Giovanni-Addolorata Hospital, Rome

Background: Triple negative breast cancer (TNBC) has an aggressive clinical behavior, with a poorer prognosis compared to other subtypes. Recently, tumor infiltrating lymphocytes (TILs) have been proposed as a predictive biomarker for a better clinical outcome and pathological response (pR) after neoadjuvant chemotherapy (NACT) in TNBC. These data confirm the role of the immune system in the neoplastic progression and in the response to therapy.

Design: We performed a retrospective analysis of 54 pre-NACT biopsies of TNBC, and compared both the percentage of stromal TILs and the degree of PD-L1 expression with the extent of pR to standard NACT.

Results: A pathological complete response (pCR) was achieved in 35% of cases. Univariate analysis showed a significant association between PD-L1 expression in ≥25% of neoplastic cells and the achievement of a pCR (p=0.024). A pCR was also significantly more frequent in cases showing ≥ 50% stromal TILs (p=0.029). In the multivariate analysis only PD-L1 expression on tumor cells remained significantly associated with pCR (OR = 1,13; 95% CI 1,01-1,27).

Conclusions: According to our results a cut-off value of PD-L1 in ≥25% of tumor cells predicts pCR in TNBC and to our knowledge our study is the first dealing with an exclusive population of TNBC cases. A possible explanation for our observation is that PD-L1 expression could be associated with a subpopulation of TNBC with a more aggressive behaviour, likely to respond to chemotherapy. Further studies with larger number of cases are warranted to confirm our findings.

155 Equivocal HER2/neu FISH Results in Breast Cancer: How Frequently Do They Change on Repeat Testing?

Nina Chang[1], *Hui Chen*[2], *Aydah M Al-Awadhi*[1], *Guilin Tang*[3], *Melissa K Robinson*[3], *Bora Lim*[4], *Aysegul Sahin*[3]. [1]MD Anderson Cancer Center, Houston, Texas, [2]UT MD Anderson Cancer Center, Houston, TX, [3]MD Anderson Cancer Center, Houston, TX, [4]MD Anderson Cancer Center

Background: Fluorescent in situ hybridization (FISH) is the gold-standard method for determining HER2/neu status in breast cancer. Selection of therapeutic regimens is problematic in patients with HER2-equivocal tumors. The 2013 ASCO/CAP guidelines recommend reflex testing for all HER2-equivocal tumors, and some remain in the equivocal category despite reflex testing. In this study, we determined the frequency of change in HER2 status upon repeat FISH testing.

Design: We retrieved records for equivocal HER2 FISH tests performed on breast cancer specimens (n=85) from Jan. 2015 to June 2017. Of these, 65 patients had an equivocal result with no known prior treatment with HER2-targeted therapy and no prior positive FISH result at our institution. The index equivocal and concurrent FISH results were reviewed for each patient, and data, including HER2 immunohistochemistry (IHC) results, were reviewed for all associated pathology specimens.

Results: The 65 patients had a total of 96 FISH tests at our institution. Thirty-seven patients had a single equivocal FISH result. In 28 patients who had 2 or more FISH tests, a repeat test performed on a different specimen or tissue block ultimately classified the HER2 status as negative in 17 cases (61%, "equivocal to negative" [EN] group), positive in 1 case (4%, "equivocal to positive" [EP] group), and equivocal in 10 cases (36%, "equivocal to equivocal" [EE] group). There were no significant differences between the EE and EN groups in the index tumor with respect to histologic grade, nuclear grade, percentages of positive ER and PR staining, Ki67 proliferation index, HER2/CEP17 ratio, and average HER2 and CEP17 copy numbers (Table 1). Fifty-six of the index tumors had associated HER2 IHC, of which 27/56 (48%) were equivocal (Table 2). Taking into account both HER2 IHC and repeat FISH results, 21/59 cases (36%) remained equivocal after additional testing with HER2 IHC and/or repeat FISH. Repeat FISH testing identified 1 HER2-positive case that was initially equivocal by both FISH and IHC (Table 1).

Table 1: Results for the index and repeat FISH tests in tumors that changed HER2 category.

FISH group	FISH result	HER2/CEP17 ratio, median (range)	Average HER2 copy #, median (range)	Average CEP17 copy #, median (range)	Cells counted, mean
EE (N =10)					
Index	Equivocal	1.5 (1.1-1.9)	4.55 (4.1-5.4)	2.8 (1.9-4.0)	120
Repeat	Equivocal	1.4 (0.9-1.95)	4.75 (4.0-5.69)	3.02 (2.7-5.2)	114
EN (N = 17)					
Index	Equivocal	1.31 (1.1-1.74)	4.59 (4.0-5.6)	3.3 (2.69-4.5)	120
Repeat	Negative	1.24 (0.7-1.7) *	3.2 (1.83-3.9) *	2.6 (1.7-4.15) *	64
EP (N = 1)					
Index	Equivocal	1.6	4.5	2.9	120
Repeat	Positive	2.0	5.6	2.7	120

*Significant difference between the EE and EN groups by the Mann-Whitney U Test, $p < 0.01$.

Table 2: HER2 IHC results for the index tumors reported as equivocal by FISH.

FISH Group	IHC				No IHC	Total
	0	1+	2+	3+		
With repeat FISH						
EE	2	3	3*	0	2*	10
EN	1	8	7	0	1	17
EP	0	0	1	0	0	1
No repeat FISH						
	3	11	16*	1	6	37
Total	6	22	27	1	9	65

*Cases remaining equivocal after IHC and/or repeat FISH testing.

Conclusions: Repeat FISH testing in patients with equivocal FISH results may help clarify treatment selection. Initially FISH-equivocal breast cancers changed categories in about 2/3 of cases on repeat FISH testing, and repeat FISH testing decreased the number of tumors remaining in the equivocal category compared with using additional IHC alone. Furthermore, utilizing only IHC and one FISH test may not identify all patients who could benefit from HER2-targeted therapy.

156 HER2 Staining Intensity Shows Prognostic Impact on Patients with HER2-Positive Invasive Breast Cancer

Yen-Ying Chen[1], *Chih-yi Hsu*[2]. [1]Taipei City, [2]Taipei Veterans General Hospital, Taipei, Taiwan

Background: Human epidermal growth factor receptor 2 (HER2) is a key marker for breast cancer, and HER2-targeted therapy has improved the prognosis of patients with HER2 overexpressed breast cancer. HER2-targeted therapies will be recommended for both HER2 IHC 3+ tumors and HER2 IHC 2+ tumors with reflex ISH positivity. However, the prognoses may be different in these two groups of patients. We hypothesized that patients with HER2 IHC 3+ tumor would have better response to anti-HER2 therapy due to higher HER2 protein expression, and their prognosis would be better than those with HER2 IHC 2+ tumor. This study aimed to evaluate whether the degree of HER2 IHC positivity affected the outcome of early breast cancer.

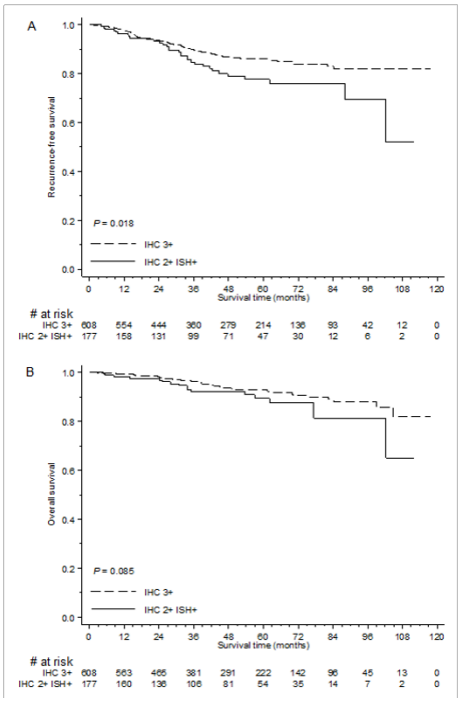
Design: Clinicopathological information of 785 consecutive cases of HER2+ early breast cancer were retrieved from the medical records. The median follow-up time was 45.5 months. The results of ER, PR and HER2 immunohistochemistry and HER2 ISH were evaluated according to current guidelines. Reflex ISH test using fluorescence in situ hybridization was performed for cases with an equivocal HER2 IHC result (score 2+). Additional HER2 ISH test was performed on 64 randomly selected cases with a positive HER2 IHC result (score 3+) to evaluate the copy number of HER2 gene. The results were correlated with the survival of patients.

Results: Hormonal receptor positivity, single anti-HER2 agent treatment and tumor recurrence occurred more frequently in HER2 IHC 2+ cases than in HER2 IHC 3+ cases. Both HER2 signals and HER2/CEP17 ratio of the HER2 IHC 3+ group were significantly higher than those of the HER2 IHC 2+ group ($p < 0.001$). Recurrence-free survival of cases with HER2 IHC 3+ tumor was significantly better than that of cases with HER2 IHC 2+ tumor (p = 0.018). However, the difference of overall survival between cases with HER2 IHC 3+ tumors and those with score 2+ tumors did not reach statistical significance (p = 0.085). Multivariate analyses revealed that hazard ratios of cases with HER2 IHC 3+ was significant smaller than those with HER2 IHC 2+ in both RFS ($p = 0.001$) and OS ($p = 0.007$) with adjustment of age, stage, hormonal receptor status, and anti-HER2 treatment.

Table 1. Multivariate Cox regression analysis of prognostic factors in patients with HER2 positive breast cancer

	Recurrence-free survival		Overall survival	
	Hazard ratio	p	Hazard ratio	p
Age (years)	1.020	0.021	1.030	0.010
Stage				
I	1		1	
II	2.507	0.002	3.260	0.008
III	5.949	< 0.001	7.794	< 0.001
Hormonal receptor				
Negative	1		1	
Positive	0.568	0.008	0.426	0.005
Anti-HER2 therapy				
None	1		1	
Single	0.509	0.003	0.489	0.015
Dual	0.386	0.010	0.131	0.006
HER2 IHC				
2+	1		1	
3+	0.469	0.001	0.424	0.007

IHC, immunohistochemistry



Conclusions: The intensity of HER2 IHC provided prognostic information for HER2-positive breast cancer. The prognosis of HER2 IHC 3+ cases was significantly better than that of HER2 IHC 2+ and ISH amplified cases.

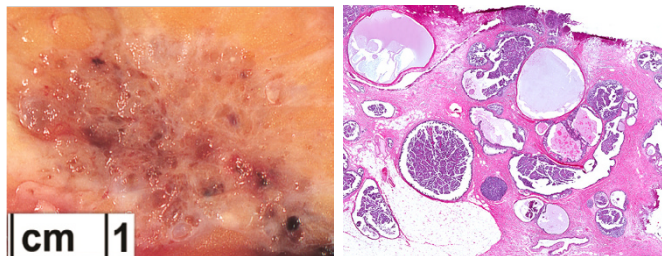
157 Mammary Juvenile Papillomatosis/"Swiss Cheese" Disease (MJP/SCD): Clinicopathologic Study of 121 Cases Reiterates Need for Extended Follow-Up

Esther Cheng[1], *Timothy D'Alfonso*[2], *Ami Patel*[3], *Kartik Viswanathan*[4], *Syed Hoda*[5]. [1]New York Presbyterian Hospital - Weill Cornell Med, New York, NY, [2]Weill Cornell Medical College, New York, NY, [3]New York Presbyterian Hosp. Weill Cornell Medicine, New York, NY, [4]New York Presbyterian Hospital - Weill Cornell Medicine, [5]Weill Cornell, New York, NY

Background: MJP/SCD, as characterized by Rosen et al (*Am J Surg Pathol* 1980;4:3; *Cancer* 1982;49:2591; *Cancer* 1985;55:1345; *Am J Clin Pathol* 1990;93:599), is a rare disease which manifests as a distinctive spectrum of various proliferative and non-proliferative changes. The clinical implications of MJP/SCD have not been further elucidated since the reports of Rosen et al - except in much smaller series.

Design: Cases diagnosed as MJP/SCD over a 16.5 year period (2001-2017) were reviewed. Pathological diagnosis was confirmed in each case. Key clinical and pathological data were analyzed.

Results: 121 MJP/SCD cases were studied. Mean age at diagnosis was 34.1 (range 13-77). 120 were female. 4 were bilateral, 59 involved left breast, 58 right. 94 (78%) presented as a palpable mass, and 16 (13%) with imaging abnormality. Grossly, each specimen was multicystic (Fig.1). Microscopically, all showed variable papillary and florid hyperplasia, cysts with foamy histiocytes, cystic apocrine hyperplasia and apocrine cysts (Fig.2). At presentation, MJP/SCD was associated with invasive carcinoma (ca) (1, 0.8%). In a mean follow-up of 113 months (range: 1-212), in 113 cases, there were 2 ipsilateral invasive ductal ca (including one in a male, reported: *Int J Surg Pathol* 2017;25:536), 2 ipsilateral ductal carcinoma in situ, and 6 ipsilateral recurrent MJP/SCD. 1 patient had Cowden and another Proteus syndrome. There was family (first degree) history of breast ca in 6/121 (5%) patients.



Conclusions: Of the 121 MJP/SCD patients, 3% (4/121) developed ca (2 invasive), and 5% (6/121) had recurrence of disease. Hamartomatous syndromes were associated in 2 cases. In 5% (6/121) there was history of breast ca in first degree relatives. This study confirms the previously reported clinical implications of MJP/SCD and reiterates the need for extended follow-up.

158 Methodology Used to Determine HER2 Status Correlates with Response to Neoadjuvant Chemotherapy in HER2 Positive Breast Cancer

Esther Cheng[1], *Patrick McIntire*[2], *Timothy D'Alfonso*[3], *Paula Ginter*[4]. [1]New York Presbyterian Hospital - Weill Cornell Med, New York, NY, [2]Weill Cornell Medicine - New York Presbyterian Hospital, New York, NY, [3]Weill Cornell Medical College, New York, NY, [4]Weill Cornell Medicine, New York, NY

Background: Breast cancer patients with tumors which overexpress HER2 protein by immunohistochemistry (IHC) and/or show *HER2* gene amplification by fluorescence in situ hybridization (FISH) are eligible for anti-HER2 therapy. There is evidence that anti-HER2 therapy activity is higher in patients with IHC positive (3+) tumors than IHC equivocal (2+) tumors in the adjuvant setting. Anti-HER2 therapy may also be given with neoadjuvant chemotherapy (NAC). We sought to compare the patterns of response between tumors that were deemed HER2-positive by IHC (3+) versus those that were positive by only FISH (IHC not 3+).

Design: This study included cases of HER2+ invasive breast carcinoma treated with NAC and neoadjuvant anti-HER2 targeted therapy. The original HER2 immunostained pre-NAC core biopsy slides were reviewed. Information regarding ER status and HER2 FISH ratios were obtained from the electronic chart. HER2 IHC and FISH were assessed according to the current American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) recommendations. Statistical analysis was performed using two-tailed Fisher's exact test.

Results: 84 patients were identified with HER2-positive tumors in pre-NAC core biopsies, including 66 that had HER2+ tumors by IHC (3+) and 18 with FISH-positive tumors (IHC 1 or 2+). Overall, 33 (39%) showed pathologic complete response (pCR). pCR was significantly more frequent in patients with IHC 3+ tumors (30/66 [45.5%]) compared with patients with FISH-positive (IHC 1+ or 2+) (3/18 [16.7%] (p=0.03). The HER2/CEP17 FISH ratios for the cases that were not IHC positive ranged from 1.3 to 8.16 (median: 3.34). Among the IHC 3+ tumors, 51.5% (34/66) were ER positive and 48.5% (32/66) were ER negative. There was no statistically significant difference in response to NAC in ER+/IHC+ versus ER-/IHC+ tumors (p>0.05). Among the FISH-positive (IHC 1+ or 2+) tumors, 72.2% (13/18) were ER positive and 27.7% (5/18) were ER negative. There was no statistically significant difference in response to NAC in ER+/FISH+ versus ER-/FISH+ tumors (p>0.05).

Conclusions: We found that in setting the of NAC, HER2 IHC positive tumors respond more favorably than HER2 FISH positive, IHC negative or equivocal cases. We found no difference in response to NAC regardless of ER status in the IHC positive or FISH positive cases. These findings suggest that the methodology utilized to determine HER2 positivity may predict response to NAC.

159 Discordant Oncotype DX Hormone Receptor and HER2 Results Infrequently Affect Adjuvant Treatment Decisions for Breast Cancer Patients

Esther Cheng[1], *Paula Ginter*[2], *Syed Hoda*[3], *Timothy D'Alfonso*[4]. [1]New York Presbyterian Hospital - Weill Cornell Med, New York, NY, [2]Weill Cornell Medicine, New York, NY, [3]Weill Cornell, New York, NY, [4]Weill Cornell Medical College, New York, NY

Background: Oncotype DX *Recurrence Score* (ODX) routinely reports expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) by RT-PCR. Discordant results between ODX and those obtained by immunohistochemistry (IHC) and/or fluorescence in situ hybridization (FISH) can cause uncertainty for clinicians regarding optimal adjuvant treatment, particularly with cases of ER and HER2 discordance. We studied the frequency of biomarker discordance and examined how it affects patient management.

Design: A search was performed (1/2011-7/2017) for patients with invasive breast carcinoma with ODX results and ER, PR, and HER2 IHC and/or FISH performed at our institution. Discordance was defined as positive with one test (ER/PR: ≥1% cells; HER2: 3+ or FISH amplified; ODX: ≥ cutoff of expression units) and negative with the second test. Slides from ER and HER2 discordant cases were reviewed and clinical data were retrieved.

Results: The study group included 743 cases. Overall, 117 (15.7%) were discordant for at least one receptor. PR discordance was most frequent, seen in 97 (13%) cases, including 84 (11.3%) in which PR was the only discordance. ER was discordant in 23 (3%) cases, all of which were IHC-positive and ODX-negative. Weak ER staining intensity or staining in ≤10% of cells was seen in 11 (47.8%) cases. The remaining 12 cases showed >10% cells staining with moderate-strong intensity. Most ER discordant cases were Grade 3 (20/23 [86.9%]) and 22/23 (95.7%) had high-risk Recurrence Scores. Of 20 ER discordant patients with follow-up, 18 (90%) received adjuvant hormonal therapy and 2 (10%) patients received chemotherapy only.

HER2 discordance was seen in 7 (0.9%) cases. All HER2 discordant cases were IHC or FISH-positive and ODX-negative. In 6 cases, FISH was positive and IHC was 1+ or 2+. One case was IHC 3+ and FISH-equivocal. Five of 7 patients with IHC/FISH-positive, ODX-negative tumors received anti-HER2 therapy. Additionally, 16 (2.2%) cases were FISH-equivocal and were HER2-negative by ODX, 3 of which received anti-HER2 therapy. Three (0.4%) cases were ODX-equivocal and IHC-negative (0/1+), and none received anti-HER2 therapy.

Conclusions: Discordance between ODX and IHC/FISH for ER and HER2 is uncommon, occurring in less than 4% of cases in this study. Clinicians tend to rely on IHC and FISH over ODX hormone receptor and HER2 results when making adjuvant treatment decisions.

160 Metastatic Breast Cancer Simulating Well-Differentiated Neuroendocrine Neoplasms of Visceral Organs

Jeffrey Cloutier[1], *Elizabeth D Thompson*[2], *Ashley Cimino-Mathews*[3], *Andres Matoso*[3], *Pedram Argani*[4]. [1]Stanford University, Stanford, CA, [2]Johns Hopkins Medical Institutions, Baltimore, MD, [3]Johns Hopkins Hospital, Baltimore, MD, [4]Johns Hopkins Hospital, Baltimore, MD

Background: Metastatic breast carcinoma (MBC) mimicking visceral well-differentiated neuroendocrine neoplasms has not previously been systematically studied.

Design: We identified five consultation cases originally submitted as neuroendocrine neoplasms but which were found to be MBC on subsequent review. In addition, we created and studied tissue microarrays (TMAs) containing primary breast carcinomas (PBC) and matched hematogeneous MBC from 20 unrelated patients (>300 total tumor spots) to determine the frequency of synaptophysin and chromogranin immunohistochemical (IHC) labeling in MBC.

Results: All consultation patients were female (age range, 39-74 years). All 5 neoplasms demonstrated nested architecture and relatively uniform nuclei which suggested neuroendocrine differentiation. Four of these patients had a known history of breast cancer (remote in 3 and concurrent in 1), but the metastases (3 liver, 1 lung) labeled for chromogranin and/or synaptophysin and thus were originally diagnosed as neuroendocrine neoplasms. In a fifth case, a liver metastasis in a patient with no known history of breast cancer but with a known pancreatic endocrine neoplasm was originally thought to be of pancreatic origin: an occult concurrent PBC was subsequently identified as the source. On further IHC, all five metastases were diffusely (>90%) and strongly positive for estrogen receptor (ER) (5/5 cases) and GATA3 (4/4 cases). Three patients had previously received ineffective treatment for neuroendocrine carcinoma. Based upon the consultation diagnosis, all four patients with follow-up received hormone therapy, which was effective in three. In the TMA cohort of matched PBCs and hematogenous MBCs, chromogranin/synaptophysin IHC labeling was absent in 85% of cases, decreased in the MBC in 10% of cases, and increased in the MBC in 5% of cases.

Conclusions: While neuroendocrine differentiation is uncommon in breast cancer and does not commonly increase in metastases, MBC with neuroendocrine differentiation should be considered in patients with visceral neuroendocrine neoplasms of unknown primary site. Accurate diagnosis permits effective therapy. Clues to the diagnosis include a remote history of PBC, permeative pattern of growth, absence of "salt and pepper" chromatin, and increased mitotic rate. Diffuse IHC labeling for ER and GATA3 establishes the correct diagnosis.

161 Beta-Catenin Activation in Breast Cancer: Its prognostic role and association with ER signaling in ER positive cancers

Kimberly Cole, Iowa City, IA

Background: Beta-catenin is a known oncogene is breast cancer. Its activation has been described in association with basal-like and triple negative breast cancers. Beta-catenin is a multifuncitional protein which is present at the cell membrane in association with e-cadherin, where it plays a structural role. It is also present as part of the WNT/beta-catenin signalling pathway in the cytoplasm and nucleus. Its activation has previously been studied using immunohistochemistry, with a membranous staining pattern indicating inactive signaling and reduced/absent membranous or nuclear/cytoplasmic indicating an activated WNT/beta-catenin pathway.

			Membranous	Reduced	Absent	
	Parameter	N				P-value
			Staining	Staining	Staining	
	Tumor Stage					
			2	1	1	
	T0					
			26	36	5	
	T1	146				P=0.0002
			11	37	7	
	T2		5	12	3	
b-Catenin	T3 +T4 *Nodal Status*					
			21	43	5	
	N0					
			11	17	5	
	N1	133				P=0.2239
			3	12	0	
	N2					
			4	12	0	
	N3 *Tumor Stage*					
			3	0	0	
	T0					
			44	26	3	
		153				P=0.000007
			22	33	3	
	T2					
E-Caderin			6	8	5	
	T3+T4					
	Nodal Status		33	31	4	
	N0	135	17	16	2	P=0.9975
	N1		8	6	1	
	N2		8	6	1	
	N3					

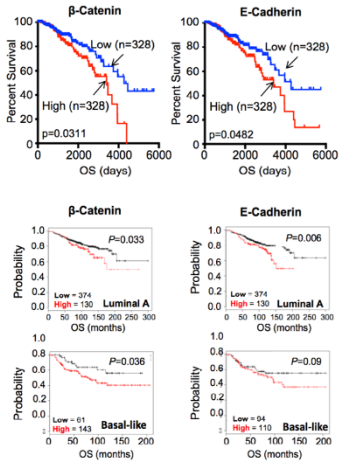
Design: We used TCGA data to evaluate the association of beta-catenin and e-cadherin protein and RNA expression with outcome. We also evaluated TCGA protein expression data to correlate high expression of beta-catenin with other proteins by intrinsic molecular subtype. Using a MCF-7 Luminal A breast cancer cell line, we looked at whether activating the WNT/beta-catening pathway is asscociated with ER pathway activity. We also evaluated the association of immunohistochemical expression of beta-catenin and e-cadherin in 163 breast cancer cases to pathologic characteristics.

Results: Both beta-catenin and e-cadherin protein expression were significantly associated with worse survival in all breast cancers and high levels of beta-catenin RNA expression were signifcantly associated with poor outcome in Luminal A and Basal-like subtypes.

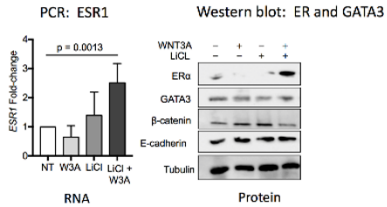
High levels of beta-catenin protein correlated with high levels of other proteins in the WNT/beta-catenin signaling pathway, e.g, Dvl and GSK, in basal-like intrinsic molecular subtype. However, in Luminal A cancers, high levels of beta-catenin protein were associated with high expression of ER-alpha and GATA-3 protein, which is a transcription factor in the ER signalling pathway.

By immunohistochemistry, reduced and or absent expression of beta-catenin and e-cadherin is associated with advanced stage tumors.

Luminal A cell lines (MCF-7) that were activated with WNT ligands and deactivation of inhibitory factors showed increased ESR1 RNA expression py PCR and increased ER-alpha and GATA-3 protein expression by western blot, see Figure 2.



ER Positive Breast Cancer Cell Line to Measure ESR1 Expression



Conclusions: Beta-catenin protein and RNA expression is assocated with poor survival in Luminal A and Basal-like breast cancers. Beta-catenin protein is associated with activation of the WNT pathway in basal-like cancers, and is correlated with ER pathway activation in Luminal cancers.

162 CIBERSORT Analysis of TCGA Identifies Subgroups with Better Outcomes in Triple Negative Breast Cancer

Kelly Craven[1], *Yesim Gokmen-Polar*[1], *Sunil Badve*[2]. [1]Indiana University School of Medicine, Indianapolis, IN, [2]Indiana Univ/Medicine, Indianapolis, IN

Background: Studies from our group, amongst others, have indicated that the presence of tumor infiltrating lymphocytes (TILs) in triple negative breast cancer (TNBC) is associated with better prognosis in chemotherapy treated patients. A meta-analysis of multiple clinical trials suggested that 20% might be good cutoff for high and low TILs. The basics of these associations are not very clear and need further investigation. Using data from The Cancer Genome Atlas (TCGA), we sought to identify the utility of this cutoff and relevance of specific subpopulations of TILs to outcomes.

Design: The TCGA Breast Cancer cohort (BRCA) was analyzed to identify TNBC cases with survival data and H&E images. The cases were classified as low and high TILs based on the 20% cut-off. CIBERSORT analysis was performed using the RNA-Seq gene expression data to identify subtypes of immune cells. The presence of high and low TILs and the type of immune cells were correlated with overall survival (OS) and disease free survival (DFS). P values were calculated using the log-rank method.

Results: Approximately 70% of 103 TNBC cases with available survival data and H&Es were classified as low TILs (<20%). Survival analysis did not identify any differences in overall or disease free survival between the two groups, even when correcting for various co-variates like age and stage. CIBERSORT analysis demonstrated a wide distribution of CD8 T cells or CD4 memory activated T cells in TNBC cases. Cases with high CD8 T cell infiltrate had a better overall survival (p-value 0.013). Better disease free survival was noted in cases with high CD4 activated memory T cell infiltrate (p-value 0.034).

Conclusions: High and low TILs based on the 20% cutoff did not have prognostic relevance. Identification of subtype of immune response will serve as the basis for further investigation into what molecular characteristics might be influencing this dichotomy of the immune response.

163 The Health Care Value of Oncotype DX® for Patients with Recurrence Scores of 10 or Less: A Value Based Pathology Study of Tumor Biology with Outcomes

David Dabbs[1], *Beth Clark*[2], *Kate Serdy*[3], *Rohit Bhargava*[1], *Sherie Smalley*[4], *Stephen Perkins*[5], *Adam M Brufsky*[6]. [1]Magee-Womens Hospital of UPMC, Pittsburgh, PA, [2]University of Pittsburgh, Pittsburgh, PA, [3]Pittsburgh, PA, [4]West Sacramento, CA, [5]UPMC, [6]University of Pittsburgh, Pittsburgh, PA

Disclosures: Adam Brufsky: *Consultant*, Agendia, Biotheranostics, Myriad

Background: Pathologists provide expert tissue assessment of breast cancer, yet are not involved with utilization of gene expression profile tests (GEP). The specific aims of this study are to detail breast tumor biologic characteristics of tumors with Oncotype DX® Recurrence Scores (RS) of 10 or less, and to determine if pathologists can prospectively identify breast cancers which do not require chemotherapy*.

Design: Onco*type* DX® cases with RS ≤ 10 from 2005-2010 comprised 441/2594 (17%) of patients, who had 5 years follow-up and treated with endocrine therapy. Tumors were analyzed for type, Nottingham grade (NG), mitotic score (MS) and hormone receptor content (H-Score). Data from this study was used as a validation set to prospectively identify tumors with low recurrence scores.

Results: Patients were age 33-92 with five-year breast cancer specific survival of 99.7% on endocrine therapy alone. Tumors in this group have unique pathology: 148 tumors (34%) had a MS1 and were tubular, cribriform, papillary, mucinous or classic lobular. The remaining 293 tumors (66%) were ductal, no special type (NST), and 261/293 (89%) of these had a MS of 1/NG2. The combined 148 special and NST tumors (93% of cases) had median H-Scores of ER (280)/PR (210) (Allred scores each ≥ 6. (Of the remaining cases, 10 (3%) had a MS of 2/NG2, 18 (6%) had MS of 2/NG3 and four (1%) were MS3/NG3. Two patients had distant recurrence, one died of breast cancer.

The prospective study arm has accrued 128 cases to date. Using MS1 and ER H-Score ≥ 200, PR H-Score ≥ 150, the sensitivity/specificity for identifying RS of ≤ 10 is (51%/90%). Of the 23 false negative cases not correctly identified as RS ≤ 10, 19 (83%) had RS ≤18 and 4 cases (17%) had RS of 19-22. Importantly, the selection criteria correctly identified all cases below the imputed chemotherapy cutpoint from TAILORx (RS<25*).

Patients aged 33-92 had five-year breast cancer specific survival of 99.7% on endocrine therapy alone. Tumors in this group have unique pathology: 148 tumors (34%) had a MS1 and were tubular, cribriform, papillary, mucinous or classic lobular. The remaining 293 tumors (66%) were ductal, no special type (NST), and 261/293 (89%) of these had a MS of 1/NG2. The combined 148 special and NST tumors (93% of cases) had median H-Scores of ER (280)/PR (210) (Allred scores each ≥ 6. (Of the remaining cases, 10 (3%) had a MS of 2/NG2, 18 (6%) had MS of 2/NG3 and four (1%) were MS3/NG3. Two patients had distant recurrence, one died of breast cancer. The prospective arm has accrued 128 cases. Using MS1 and ER H-Score ≥ 200, PR H-Score ≥ 150, the sensitivity/specificity for identifying RS of ≤ 10 was (51%/90%), and was (90%/90%)for RS≤18. Importantly, the selection criteria correctly identified all cases below the imputed chemotherapy cutpoint from TAILORx (RS<25*).

Conclusions: (1) Pathologists add great value to triage breast GEP. (2) Pathologists identify low grade tumor biology with high sensitivity and high specificity for those cases which do no not require chemotherapy* (RS<18) using mitotic score and hormone receptor content. (3) Onco*type* DX® lacks health care value for tumors with pathologically identified low biological aggressiveness.

The performance of the 21-gene assay standard cut points of 18 and 31 in HR+,HER2- invasive breast cancer while waiting for TAILORx mid-range recurrence results. J Clin Oncol 35, 2017 (suppl. Abstract 537).

164 Comparison of Oncotype DX Breast Recurrence Score® Values from Repeat Testing of the Same Primary Invasive Breast Cancers.

Puja Dadhania[1], *Anson Tharayanil*[1], *Jerome V Braun*[2], *Samson Peter*[2], *Carolyn Mies*[3]. [1]Genomic Health, Inc., Redwood City, CA, [2]Genomic Health, Inc., [3]Genomic Health, Incorporated, Redwood City, CA

Disclosures: Puja Dadhania: *Employee*, Genomic Health, Inc.
Anson Tharayanil: *Employee*, Genomic Health, Inc.
Carolyn Mies: *Employee*, Genomic Health, Inc.

Background: The Oncotype DX® test uses RT-PCR to produce the Breast Recurrence Score® (RS) result which has been validated to predict the risk of distant recurrence and benefit of chemotherapy in early-stage, ER-positive invasive breast cancer (IBC) patients (pts). Infrequently, physicians will request repeat Oncotype DX® testing under our quality assurance program. Reasons for retests include: discordances in tumor biomarker assessment by IHC and RT-PCR (Gene Discordance), concern that a suboptimal sample was tested

(Suboptimal Tested), discordance between the RS value and grade or other pathological features (Pathological Discordance), concern the tested sample was not appropriately microdissected (Suboptimal Dissection) and miscellaneous. To examine the reproducibility of the assay, we compared RS values derived from repeat Oncotype DX tests on the same or different samples from the same primary IBC.

Design: Between 6/1/2013 – 6/24/2016, 92 of 233,946 IBC pts had one or more retests on either the same block (Intrablock), an adjacent block (Intracase), or a block from a different surgical case (Intercase) from the same primary IBC. A central pathologist examined paired H&E slides to compare morphology and nuclear grade between tested samples. We assessed concordance between paired RS values using scatterplots, Pearson correlation coefficients (PC), and other summary statistics.

Results: For the 98 paired samples, the mean difference between paired RS values was -2.6 (95% CI -4.2, -0.9) and Pearson correlation (PC) was 0.9. Tables 1 and 2 display results by retest reason and retest type. 94/98 paired samples were morphologically similar.

Table 1.

Retest Reason	Retests (N)	PC	Magnitude of Mean difference of RS values
Gene Discordance	28	0.86	5.6
Suboptimal Tested	39	0.82	1.6
Pathological Discordance	14	0.96	0.9
Suboptimal Dissection	7	0.95	1.1
Miscellaneous	10	0.98	1.3

Table 2.

Retest Type	N	PC	Magnitude of Mean difference of RS values
Intrablock	19	0.97	0.1
Intracase	19	0.93	1.1
Intercase	60	0.88	3.9

Conclusions: Consistent with prior studies, RS values of different samples from the same primary IBC were highly concordant. In most cases, RS values obtained from repeat testing did not produce clinically actionable changes. To ensure optimal patient care, clinicians who encounter potentially discordant results should investigate until a resolution is achieved. The local pathologist plays a critical role in submitting the most representative tumor sample for Oncotype DX testing.

165 Malignant Osseous Differentiation in Breast Malignancies: A Mayo Clinic Experience

Nooshin Dashti[1], *Saba Yasir*[2]. [1]Mayo Clinic, Rochester, MN, [2]Mayo Clinic

Background: Malignant osseous differentiation (MOD) can be seen in various types of breast malignancies. Therefore MOD when seen in core needle biopsies (CNB) poses a significant diagnostic challenge. There is limited data available in this challenging area. We conducted this study to provide a comprehensive data by evaluating osteosarcomatous differentiation in breast in various malignancies and clinical settings.

Design: Our institutional and consult archive from 1992- 2017 was systematically searched for breast cases coded as "osteosarcoma" or "osteoid". All patients who underwent excisions were included, with the exception of some post-radiation sarcomas and metastatic disease cases, that were biopsies. All available radiologic and histologic material was reviewed.

Results: A total of 31 female patients were identified. Of those 23 had resections and 8 underwent CNBs. Malignant osseous differentiation was identified in five different disease categories. 1. Metaplastic carcinomas with prominent osseous differentiation (6 of 31 cases; 19%). One of the 6 patients had osteosarcomatous metastasis to the lungs. The age ranged from 40-91 years (mean: 68 years). 2. Extraskeletal osteosarcoma (EOS) (13 of 31 cases; 42%) with age range from 45-99 years (mean: 76 years). All EOS cases were completely negative for broad spectrum, low and high molecular weight keratins. 3. Only one patient had malignant phyllodes tumor with heterologous osseous elements (age: 47 year). 4. One patient had metastatic osteosarcoma to the breast from a known femur primary (age: 21 years). 5. Post radiation osteosarcoma involving the breast and chest wall (10 of 31, including 7 biopsies and 3 resections, 32%). The age ranged from 30-71 years (mean: 66 years). Histologic review of subset of cases showed typical morphology of high-grade osteosarcoma with neoplastic osteoid elements surrounding highly pleomorphic cells in a lace like fashion. Mineralized osteoid was not identified. Some tumors showed undifferentiated spindle cell sarcoma pattern with prominent giant cells.

Conclusions: Our data showed a wide spectrum of breast diseases with MOD. On CNBs, MOD raises the possibility of metaplastic carcinoma, phyllodes tumor, EOS or metastasis. Definitive diagnosis should only be made on resection and after clinical correlation. Our data showed a high percentage of EOS on resection. A subset of cases in this group may represent metaplastic carcinoma or phyllodes tumor completely replaced by malignant osseous differentiation.

166 Suboptimal Concordance in Re-Testing results of Triple Negative Breast Carcinoma (TNBC) Cases Among Laboratories

Jose R De Jesus[1], *Emmanuel Agosto-Arroyo*[2], *Laila Khazai*[1], *Marilin Rosa*[3]. [1]Moffitt Cancer Center, [2]Moffitt Cancer Center, Tampa, FL, [3]Moffitt Cancer Center, Tampa, FL

Background: Estrogen (ER) and progesterone (PR) receptors and Human epidermal growth factor receptor-2 (HER2) are the prototypical tumor markers with an immediate impact on systemic treatment decisions on patients with breast carcinoma. TNBC patients do not benefit from hormonal or HER2 targeted therapies and surgery and/or chemotherapy remain the only treatment option. Neoadjuvant chemotherapy is often offered to TNBC patients because studies have consistently reported higher response rate in TNBC than non-TNBC and pathologic complete response (pCR) has been shown to predict improved long-term outcomes for TNBC. Therefore, accurate ER, PR, HER-2 status is of upmost importance in this setting.

Design: To evaluate the degree of concordance in ER, PR and HER2 testing results, we analyzed our data of TNBC review cases in which

these markers were repeated at our institution from January 2014 to December 2016. Discrepancies were classified as major and minor according to their impact in patient care, including treatment selection.

Results: Five hundred sixty review cases were tested during the study period, 113 cases were TNBC and were included in the study. Thirty-nine discrepancies were identified (35%), 32 were major and 7 were minor discrepancies. All minor discrepancies consisted of HER2 changes from negative to equivocal or vice versa on IHC. All these cases were subsequently confirmed as negative by dual in-situ hybridization (DISH). Major discrepancies included changes in ER and/or PR from negative to low-positive (positivity between 1-10%).

Conclusions: Our study reveals a 35% discrepancy rate (39/113) in TNBC retested at our institution. More common and significant discrepancies involved ER and PR results. Since patients with only 1% ER, PR positivity have different treatment options and are eligible for hormonal therapy, re-testing of TNBC cases before starting definitive therapy is recommended.

167 SOX10 is Frequently Expressed by Triple-Negative (Basal-Like) Breast Cancer: Diagnostic Pitfall and Opportunity

Neha Dhungana[1], *Jon Thomason*[2], *Stephanie Chen*[2], *Andrew Bellizzi*[2]. [1]University of Iowa Hospitals and Clinics, [2]University of Iowa Hospitals and Clinics, Iowa City, IA

Background: The transcription factor SOX10 is a marker of melanocytic and nerve sheath differentiation. It is also expressed by myoepithelial cells and related tumors. We recently encountered a poorly differentiated breast cancer in which diffuse, strong SOX10 expression led to diagnostic confusion with melanoma. Triple-negative/basal-like breast cancers often express myoepithelial markers (e.g., p63, CK5/6), and they may be GATA-3 weak or negative. We hypothesized that SOX10 expression would be enriched in this class of tumors.

Design: Tissue microarrays were constructed from 199 breast cancers (triplicate 1 mm cores). SOX10 immunohistochemistry (IHC) was performed with expression evaluated in terms of intensity (0, 1+, 2+, 3+) and extent (0-100%) with an H-score (intensity*extent) calculated. Vital status and results of clinical ER/PR/HER2 testing were recorded with an intrinsic subtype (i.e., luminal A, luminal B/HER2-, luminal B/HER2+, Erb-B2 overexpression, basal-like) inferred according to 2013 St. Gallen Criteria. GATA-3 IHC had been previously performed. Mann-Whitney and Fisher's exact tests were used with $p<0.05$ considered significant.

Results: SOX10 expression was seen in 59% (59/100) of basal-like, 13% (5/39) of Erb-B2 overexpressing, and 0% of luminal A (23), luminal B/HER2- (33), and luminal B/HER2+ (4) cases. Among basal-like cancers, 47% co-expressed SOX10 and GATA-3, 34% were GATA-3+/SOX10-, 12% were GATA-3-/SOX10+, and 7% were negative for both markers; thus, adding SOX10 to the results of GATA-3 increased the sensitivity for the detection of breast cancer from 81 to 93%. SOX10 expression in basal-like cancers was typically strong, and GATA-3 expression was more modest, while in Erb-B2 overexpressors, the inverse was true (see Table). SOX10+ basal-like cancer patients were more likely to be alive (61% vs. 51%), but this result did not achieve

significance (p=0.41).

Table: Comparison of SOX10 and GATA-3 Expression in ER-Negative Breast Cancer

	% SOX10+	p	Mean (Median) H-score (if +)	p	% GATA-3+	p	Mean (Median) H-score (if +)	p
Erb-B2 overexpression (n=39)	13%	<0.0001	110 (102)	<0.0001	100%	0.0017	200 (240)	0.034
Basal-like (n=100)	59%		199 (212)		81%		114 (95)	

Conclusions: Strong SOX10 expression is characteristic of basal-like breast cancer. This represents a potential pitfall, which can be exploited diagnostically to suggest a breast origin in a GATA-3-negative carcinoma.

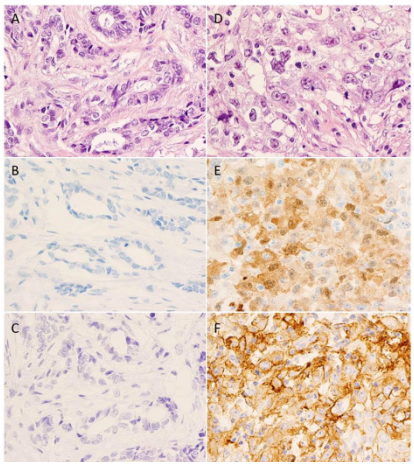
168 IDO Expression Across Breast Cancer Subtypes: An Assessment of 242 Primary and 39 Metastatic Tumors

Erik Dill[1], *Patrick Dillon*[2], *Kristen Atkins*[3], *Timothy Bullock*[4], *Anne Mills*[3]. [1]UVA Medical Center, Charlottesville, VA, [2]UVA, [3]Charlottesville, VA, [4]UVA, Charlottesville, VA

Background: Tumor expression of the immune modulatory enzyme indoleamine 2,3-dioxygenase (IDO) has been associated with immune evasion in numerous malignancies, including breast carcinomas, and may mark these cancers as susceptible to IDO inhibitor therapies. Little is known about IDO expression in tumor and peritumoral immune cells across the full range of breast cancer subtypes, and the relationship of IDO and PD-L1 expression has not been examined within breast carcinoma. We herein address IDO tumoral and immune expression in breast cancers, examine the relationship between IDO and PD-L1 expression, and investigate IDO fidelity across breast cancer primaries and metastases.

Design: IDO immunohistochemical expression was assessed in tissue microarrays containing 242 invasive primary breast cancers, 20 nodal metastases, and 19 distant metastases (4 x 0.6 mm replicate cores/case). These cases had previously been characterized for PD-L1 expression. IDO and PD-L1 were both scored by extent in the tumor cells and immune compartment.

Results: Tumor IDO staining was seen in 14% of primaries including 38% of triple-negative breast cancers (TNBC). Immune staining was seen in 14% of all primaries and 29% of TNBC. Tumoral IDO and PD-L1 co-expression was seen in 11% (26/242) of all primaries, which constituted 58% of all tumoral PD-L1-positive cases. [Figure 1: Grade 1 ductal carcinoma (A) with negative IDO (B) and PD-L1 (C); Triple-negative grade 3 ductal carcinoma (D) with strongly positive IDO (E) and PD-L1 (F)]. Immune IDO and PD-L1 co-expression was identified in 14% (33/242) of primaries, comprising 48% of all immune PD-L1-positive cases. Tumoral and immune cell IDO was conserved in 94% of matched primary/metastasis.



Conclusions: IDO expression is common among high-grade, triple-negative breast cancers. IDO expression within the tumoral and immune cell compartments is frequently associated with PD-L1 coexpression, suggesting that IDO might be a mechanism of anti-PD-1/PD-L1 immunotherapy resistance and that dual therapy may be of utility in this setting. Tumoral and immune cell IDO expression shows fidelity between primary and metastatic sites in treatment-naïve cancers, arguing against IDO as an independent driver for metastatic spread. Clinical trials are needed to assess the efficacy of IDO inhibition relative to IDO expression, as well as its possible role as a potentiator for anti-PD-1/PD-L1 immunotherapy.

169 The Ductal Carcinoma In Situ Molecular Landscape: Highlight on FAT-Family Related Genes and Potential Progression Factors.

Lounes Djerroudi[1], *Vanessa Benhamo*[2], *Elodie Girard*[2], *Laetitia Fuhrmann*, *Fabien Reyal*[5], *Alain Fourquet*, *Caterina Marchio*[4], *Arnau Montagud*[5], *François Radvanyi*[3], *Emmanuel Barillot*[3], *Ann Salomon*[5]. [1]Institut Curie, Paris, Ile de France, [2]Insitut Curie, [3]Institut Curie, [4]University of Turin, Torino, Italy, [5]Institut Curie, Paris, France

Background: Ductal carcinoma *in situ* (DCIS) is a non-obligate precursor of invasive breast cancer (IBC). Molecular mechanisms involved in the progression of DCIS to IBC remain largely unknown.

Design: We performed whole exome sequencing (WES) of 11 early lesions (5 DCIS and 6 micro-invasive DCIS (MI-DCIS)) completed by targeted sequencing, transcriptome, and immunohistochemistry (IHC) analysis of a series of patients with DCIS (n=45), MI-DCIS (n=21), IBC (n=20).

Results: WES revealed that DCIS and MI-DCIS had low mutational load (0.792 and 1.242 mut./Mb, respectively) and lower copy number alteration (CNA) compared to IBC from TCGA. Almost all cases (10/11) had potential driver gene mutations including recurrent mutations in *TP53* (2/11), *GATA3* (2/11), *PIK3CA* (2/11) and single-case mutations in other PI3K/AKT pathway genes *(AKT1, PIK3R1),* and chromatin remodelling genes *(ARID1A, ARID2, CREBBP, WHSC1L1, KMT2C). FAT3* (2/11) was one of the four recurrent mutated genes. Targeting sequencing of the validation series of DCIS (n=40) and MI-DCIS (n=6) identified mutations in *TP53* (26%) and *PIK3CA* (21.7%) at a comparable rate to IBC from TCGA. *FAT3* mutations (6.52%) occurred on intermediate grade, luminal-type DCIS. Focusing on FAT-related genes, preliminary results of integrated analysis (WES, RNAseq, IHC) revealed : significant differences in *FAT2* and *FAT3* RNA expression between DCIS and IBC; early and frequent LOH in hippo-pathway genes with TAZ nuclear localization found in 74% of DCIS, MI-DCIS and IBC; significantly lower motility-gene *ENAH* gain (9.1% [1/11] vs. 61.4%) and amplification rate (0% [0/11] vs. 13.3%) in DCIS vs. TCGA IBC ($p= 3.4\ 10^{-4}$) associated with lower ENAH RNA expression in DCIS ($p= 9.87\ 10^{-3}$); early *SCRIB* amplification (DCIS, MI-DCIS : 27.3% [3/11]; TCGA IBC : 15.2%) associated with IHC Scribble overexpression. Finally, we identified biologically relevant 1q-located pro-invasive genes with both significantly higher gain/amplification rate and RNA expression in IBC vs. DCIS (including *CAPN2, KIF26B, KIF14, CREB1, LAMC1, TGFB2, IL19, CKS1B, ASPM*).

Conclusions: Our data confirm that DCIS and MI-DCIS have low genetic alterations rates and carry driver events representative of IBC. In addition, we highlighted early involvement of FAT family and Hippo-pathway related genes in breast cancer progression. Furthermore, our analysis identified *ENAH* and 1q-located genes genomic alterations with putative driver role in DCIS invasion to be confirmed.

170 200 screen detected invasive breast carcinomas re-staged according to the AJCC 8th edition

Aoife Doyle[1], *Cecily Quinn*[2]. [1]St Vincent's University Hospital, Dublin, Ireland, [2]St Vincent's University Hospital, Dublin, Ireland

Background: In a major departure from traditional Anatomic TNM staging the AJCC 8th edition has introduced the *Prognostic Stage* to refine prognostic groups and assist personalized treatment planning. The *Prognostic Stage* combines anatomic TNM stage with histological tumor grade, tumor biomarkers and the results of the 21 gene recurrence assay (Oncotype Dx) in a subset of patients. AJCC report that 41% of patients undergo a change in stage using the *Prognostic Stage*.

Design: The study population comprised 200 consecutive patients diagnosed with invasive breast carcinoma. Disease was unifocal in 76% of patients and multifocal in 24%. Tumor grade was 1 in 22%, 2 in 56.5% and 3 in 21.5%. Lymph node status was negative in 85% of cases and positive in 15%. 85% of patients had hormone receptor positive and Her2 negative disease, 12% were Her2 positive and 3% had triple negative disease. Oncotype Dx testing, performed on 27.5% of tumours, yielded a recurrence score of less than 11 in 16 patients. Anatomic TNM staging was calculated and compared with The *Prognostic Stage*, calculated according to AJCC 8th edition using an electronic tool developed by Balint Cserni.

Results: The breakdown by Anatomic stage and Prognostic stage is demonstrated in Table 1. Applying *Prognostic Stage* resulted in 34% of patients being reassigned to a higher or lower stage compared with Anatomic TNM: 16.5% were upstaged (Table 2) and 17.5% were down-staged (Table 3).

Table 1 (n=200)

Stage	1a	1b	2a	2b	3a	3b	3c
Anatomic Stage	72.5% (145)	1.5% (3)	17.5% (35)	5.5% (11)	1.5% (3)	0% (0)	1.5% (3)
Prognostic Stage	61.5% (123)	22.5% (45)	7.5% (15)	6.5% (13)	1.5% (3)	0% (0)	0.5% (1)

Table 2 (n=33)

Anatomic Stage		Prognostic Stage	no. of cases
1a	→	1b	8.5% (17)
1a	→	2a	5% (10)
2a	→	2b	1% (2)
2a	→	3a	1.5% (3)
2b	→	3b	0.5% (1)

Table 3 (n=35)

Anatomic Stage		Prognostic Stage	no. of cases
1b	→	1a	1.5% (3)
2a	→	1b	11% (22)
2a	→	1a	2% (4)
2b	→	1b	1.5% (3)
3a	→	2b	1.5% (3)

Conclusions: Initial experience of using the *Prognostic Stage* resulted in reassignment of disease stage in 34% of patients. This staging system is more complex than traditional anatomic staging and was assisted in this study by the use of an electronic tool. *Prognostic Stage* will be implemented in the US in January 2018. On-going refinement will be required with validation of other risk assessment tools. Although retaining an anatomic basis this synthesis of anatomy and biology may lead to disparity of staging internationally due to varied access to biomarker studies and multigene assays.

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2. Sparano JA et al., *Prospective Validation of a 21-Gene Expression Assay in Breast Cancer.* N Engl J Med. 2015 Nov19;373(21):2005-14

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171 Nuclear FOXM1 (nFOXM1) Immunoexpression is Upregulated in the Progression and Grade of Breast Carcinoma, A Pilot Study Employing Automated Halo Imaging System ®

Osama Elfituri[1], *Nasma K Majeed*[2], *Haoliang Xu*[3], *Nathan Aardsma*[4], *Alexandra Barabanova, Ryan Deaton*[5], *Elizabeth L Wiley*[6], *Pradip Raychaudhuri*[5], *Frederick Behm*[5], *Grace Guzman*[7]. [1]University of Illinois at Chicago, Chicago, IL, [2]University of Illinois at Chicago Hospital and Medical Center, Chicago, IL , [3]University of Illinois-Chicago, Chicago, IL, [4]Univ. of Illinois at Chicago, Chicago, IL, [5]University of Illinois at Chicago, [6]University of Illinois at Chicago, Chicago, IL, [7]University of Illinois, Chicago, IL

Background: Breast carcinoma is the second leading cause of death among women in the USA. FOXM1, a transcription factor implicated in proliferation via control of cell cycle transition, is a potential biomarker in various cancers including liver, lung, prostate, and colon, and its activation is signaled by a switch from cytoplasmic to nuclear expression. Our aims were to characterize nuclear FOXM1 immunohistochemical expression in benign and malignant breast tissue by automated (Indica Labs Halo version 2.0.1145.21) and semi-quantitative (visual) scoring, and identify any relationship to tumor grade.

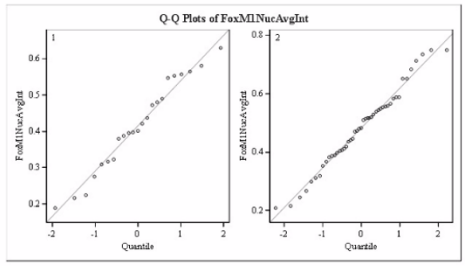
Design: Tissue microarrays (TMAs) were constructed from 70 cases of invasive breast carcinoma spanning grades 1(n=5), 2(n=18), and 3(n=47) and 40 non-neoplastic breast control tissues. TMAs were stained by FOXM1 using standard methods. nFOXM1 staining intensity was graded on a scale 0-3. TMA slides were scanned on a Leica Aperio AT2 at 200x. Indica Labs Halo version 2.0.1145.21 was used to segment spots. Multiple regions of interests were manually drawn within each core and The Halo Multiplex IHC v1.1 algorithm was used to identify epithelial nFOXM1 expression and intensity. The per cell data was exported to Microsoft Access 2013, average nFOXM1 intensity was calculated, merged with tissue diagnoses using Access, and statistical analyses was performed using SAS version 9.4. Diagnosis of each core was verified using published criteria and semi-quantitative (visual) intensity (scale 0-3) was scored by independent observers.

Results: Benign breast epithelium showed weak cytoplasmic (n=27), faint nuclear (n=4), and completely negative (n=9) staining. 70/70 malignant breast cores showed (+) nFOXM1 with a trend of increased intensity across tumor grades and significant upregulation when grades 1 and 2 were grouped versus 3 (Ttest; p=.051;Fig1). Further, there was moderate-high correlation between automated and semi-quantitative nFOXM1 scoring (.65 to .74; p<.001;Table 1).

Table 1: Pearson Correlation Coefficients
Prob > |r| under H0: Rho=0
Number of Observations

	FoxM1Int_Visual Mod	FoxM1NucAvgInt
FoxM1IntVisual Mod	1.00000 67	0.74890 < .0001 67
FoxM1NucAvgInt	0.74890 < .0001 67	1.00000 70

Figure 1: Ttest of FoxM1 Nuclear Intensity by Grade - 1+2 vs 3
High grade has more intense nuclear foxm1 than low grade(p=.051)



Conclusions: nFOXM1 expression was enhanced in breast carcinoma, and increased in high grade (grade 3) versus low grade (grades 1 and 2), suggesting its activation during carcinogenesis and its utility as a potential biomarker. Automated scoring of FOXM1 using Indica Labs Halo version2.0.1145.21 is reliable, reproducible, and time efficient in comparison to semi-quantitative scoring. Future studies with larger cohort will be necessary to determine whether automated hot spot methodology could be effectively employed.

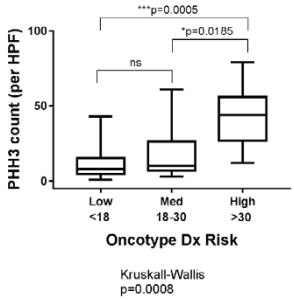
172 Phosphohistone 3 (PHH3) in breast cancer: clinical utility and predictive/prognostic value

Hala Faragalla[1], *Kiran Jakate*[2], *Teddy Nagaria*[3], *Luke Ibrahim*[4]. [1]St Michael's Hospital, Toronto, ON, [2]St Michael's Hospital, [3]University of Toronto, Toronto, ON, [4]University of Toronto, Toronto, ON

Background: Prognosis in breast cancer is inferred mostly from stage, grade and receptor status. To avoid overtreatment of early breast cancer, better tools are needed. Oncotype Dx is a genomic test which derives a recurrence score (RS) used to assess chemotherapy benefit in breast cancer patients and is heavily dependent on proliferative genes, including Ki-67. Tumor proliferation, measured as a mitotic score, is part of the current Nottingham grading system. Phosphohistone 3 (PHH3) is a protein essential for chromatin remodeling in mitosis and can be used to assess proliferation. The aim of this study is to study the correlation of PHH3 with Oncotype RS, mitotic activity index (MAI) and Ki67.

Design: Breast cancers from 71 patients with Oncotype results were studied. Immunohistochemistry was performed on whole tumor sections for PHH3 and Ki67. PHH3 counts were reported as the number of tumor cells with positive nuclear staining in 10 consecutive high power fields (40X objective) in the area with highest staining intensity. MAI was assessed in 10 consecutive high power fields in areas with highest mitotic activity after scanning the PHH3 slide to highlight these areas. Ki67 counts were reported as a percentage of tumor cells with nuclear staining. Statistical analysis was performed using SPSS Statistics software.

Results: PHH3 showed strong positive correlation with MAI (Spearman r= 0.7521 respectively), confirming its distinctive utility as proliferation marker. In all invasive ER positive and HER2 negative breast cancers, higher PHH3 and Ki67 counts were generally associated with higher Nottingham grade. Grade I tumors had significantly lower counts of PHH3 compared to Grade III tumors, (p<0.0001 respectively). Grade II tumors had lower PHH3 and Ki67 counts when compared with grade III. Furthermore, higher PHH3 and Ki67 levels were associated with high risk Oncotype RS compared to low risk RS cases (p=0.0005 and p=0.0477), while only PHH3 was able to discriminate between intermediate and high risk Oncotype RS (p=0.0185).



Conclusions: PHH3 and Ki67 both are associated well with tumor grade and MAI with higher levels associated with higher tumor grades. Higher PHH3 and Ki67 levels were associated with high risk Oncotype Dx RS, however only PHH3 was able to discriminate between medium and high risk categories. PHH3 is more accessible to manual counting, highlights hot spots for mitotic counting and shows more consistent staining results than Ki67.

173 Comparison of DCIS Recurrence Risk Prediction Models: MSK Breast Cancer Nomogram and the Oncotype DX DCIS Score

Susan Fineberg[1], *Rosemarie DiDonato*[1], *Jana Fox*[2], *Yungtai Lo*[3], *Della Makower*[1]. [1]Montefiore Medical Center, Bronx, NY, [2]Montefiore Medical Center, [3]Montefiore Medical Center

Disclosures: Jana Fox: *Speaker*, Genomic Health

Background: The Oncotype DX (ODX) DCIS Score and the Memorial Sloan Kettering (MSK) DCIS nomogram are two clinical tools used to predict local recurrence risk (RR) in patients with DCIS. The ODX is a genomic assay and the MSK nomogram is based on clinical and pathologic variables. We compared RR results in 69 patients using these two prediction tools.

Design: DCIS patients for whom ODX DCIS score had been performed (n=69) were identified by a search of the pathology database. Each patient's medical record was reviewed to determine clinicopathlogic features needed to determine RR using the online MSK nomogram (MSKCC.org/Breast/DuctalCarcinomaInSituRecurrencePage). MSK RR was calculated without radiation therapy. MSK RR was calculated both with and without Endocrine therapy (ET) for all pts. The two RR results were compared, and divided into three categories: Category 1: ODX RR higher than MSK RR both with and without ET; Category 2: ODX RR falls within MSK RR range; Category 3: ODX RR lower than MSK RR both with and without ET.

Results: The mean pt age was 65. Median (IQR) ODX RR was 13% (9-17%). Median MSK RR was 14% (12-18%) without ET and 7% (6-9%) with ET. Twenty of the 69 pts (29%) had an ODX RR which was higher than the MSK RR both with and without ET which was atleast 5% higher in 10 of 20 pts. In 8 of 69 pts (12%) ODX RR was lower than MSK RR both with and without ET, which was atleast 5% lower in only one patient. In the remaining 41 patients (59%) ODX RR and MSK RR fell within the same range when considering both with and without ET. ODX RR had a weak positive correlation with MSK RR with ET (r=0.295, p=0.014) and MSK RR without ET (r=0.285,p=0.018) . Grade 3 nuclei (p=.002), Progesterone receptor<=40% expression (p=.021) and necrosis (p=.041) were each significantly associated with a higher ODX vs MSK RR. Positive or close margins <=2mm (p=<0.0001), family history (p=.027) and presentation with clinical findings (p=0.008) were significantly associated with a higher MSK RR vs ODX RR.

Conclusions: We show a weak positive correlation between MSK RR and ODX RR RR estimates overlapped in 59% of patients. Cases showing a higher ODX vs MSK RR demonstrated aggressive biologic features. Cases showing higher MSK vs ODX RR demonstrated high risk clinical features. Since all categories of DCIS demonstrate reduced RR with radiotherapy, this study suggests that an assay incorporating both genomic and clinical factors, may most accurately demonstrate patients with the lowest RR.

174 Histomorphologic Analysis of Breast Cancers Undergoing Reflex HER2 In Situ Hybridization: A Single Institution Experience.

Livia Florianova[1], *Naama Nofech*[2], *Hala Faragalla*[3], *Kiran Jakate*[4]. [1]University of Toronto, Toronto, ON, [2]St. Michael's Hospital, Toronto, [3]St Michael's Hospital, Toronto, ON, [4]St. Michael's Hospital, Toronto, ON

Background: To expand patient access to anti-HER2 therapy, the 2013 revised ASCO/CAP guidelines lowered the threshold for positive HER2 immunohistochemistry (IHC) from 30% to 10% of tumor cells with intense complete membranous staining. Histologic subtype and grade should also be considered to decide if further testing is required in borderline cases. Equivocal IHC HER2 cases undergo reflex testing with in situ hybridization (ISH). HER2 IHC is routinely performed on invasive breast carcinoma biopsies at our institution. Since the lower positivity threshold and smaller specimen size encourages ordering ISH on a greater proportion of IHC-borderline cases, we sought to determine if tumor histomorphologic features are sufficiently predictive of ISH amplification results in order to be utilized in decision-making for ISH testing.

Design: We reviewed 101 specimens of primary and metastatic breast carcinoma that underwent HER2 ISH testing at St. Michael's Hospital (2007–2016) due to equivocal IHC results (majority of cases) or at the discretion of the pathologist. Tumor subtype, Nottingham grade and nuclear pleomorphism score were compared with HER2 ISH results (dual probe assay by fluorescence or chromogenic ISH). Cases were classified as HER2 negative or HER2 non-negative (ISH-equivocal and amplified cases) using the 2013 ASCO/HER2 recommendations. Data was categorized into contingency tables and analyzed with the Chi-Square and Fisher exact probability tests.

Results: The all-female cohort comprised of biopsies (64.4%), lumpectomies/mastectomies (27.7%) and distant metastases (7.9%) that were HER2 ISH-negative (75.2%), ISH-amplified (14.9%) and ISH-equivocal (8.9%). Proportions of Nottingham low-grade, intermediate-low (score 6), intermediate-high (score 7), and high-grade tumors were 13.9%, 25.8%, 24.7% and 35.6% respectively. While this 4-tiered classification had no association with HER2 ISH positivity, comparison of pooled low and intermediate grade tumors vs high-grade tumors showed a trend for association (21.5% vs 27.8% of HER2 amplification, p = 0.03). Nuclear pleomorphism score (high in 69.3% of cases) did not predict non-negative HER2 status. Table 1 shows ISH status by histologic subtype; all 7 mucinous, cribriform or tubular carcinomas were negative.

TABLE 1. Summary of HER2 negative and HER2 non-negative cases by tumor histology

	Invasive ductal carcinoma (IDC) NOS[1]	Invasive lobular carcinoma	IDC with lobular features	IDC with basal-like & poorly differentiated features	IDC with apocrine features	IDC with papillary & micropapillary features[2]	IDC with neuroendocrine features[2]	IDC with mucinous features	IDC, cribriform & tubular subtypes
HER2 NEGATIVE (not amplified)	40 (78.4%)	4 (80%)	7 (58.3%)	10 (83.3%)	2 (40%)	4 (66.6%)	5 (100%)	4 (100%)	3 (100%)
HER2 NON-NEGATIVE (amplified or equivocal)	11 (21.6%)	1 (20%)	5 (41.7%)	2 (16.7%)	3 (60%)	2 (33.3%)	0 (0%)	0 (0%)	0 (0%)
TOTAL[3]	51	5	12	12	5	6	5	4	3

Percentages indicate the proportion of HER2 negative and HER2 non-negative cases within each category of tumor subtype / histological variant.

[1]NOS : not otherwise specified.

[2]In 2 cases the carcinoma showed both neuroendocrine and papillary features, and was thus included in both categories.

[3]The sum of all histological subtypes and variants is 103.

Conclusions: Although low-grade breast carcinomas of certain histologic subtypes are mostly HER2 ISH negative, nuclear pleomorphism and Nottingham grade do not readily predict HER2 status.

175 PDL1/PDL2 mRNA Expression in Breast Cancer Cell Lines and Mammospheres: An in vitro Study

Marta Garcia-Escolano[1], *Araceli Garcia-Martinez*[1], *Fernando Ortiz-Martinez*[1], *Raquel Linares Fernández*[2], *Hortensia Ballester*[1], *Jose Ponce*[1], *Pascual Martinez-Peinado*[3], *J Miguel Sempere-Ortells*[3], *Gloria Peiro Cabrera*[4]. [1]University General Hospital Alicante, Spain, [2]University General Hospital ISABIAL-Alicante, Alicante, [3]University of Alicante, [4]University General Hospital Alicante, Alicante, Spain

Background: Immune responses against cancer cells are initiated after the immune system recognizes abnormally expressed proteins. Nevertheless, some tumor cells are able to escape from this immune control. PD1/PDL1 pathway has been suggested to have a key role in this mechanism of adaptive immune resistance in cancer. Several studies have described surface expression of PDL1 in tumor cells, stromal cells or both, as well as aberrant expression in human tumors. In breast carcinoma (BC), very few data from in vitro studies in BC cells and in stem cell conditions is available.

Design: In the current in vitro study we included 1 normal mammary epithelium (184A1) and 6 BC cell lines representative of all the immunophenotypes: Luminal A (MCF-7 and T47-D), Luminal B/HER2+ (BT474), HER2-enriched (SKBR3), Triple Negative/Basal-like (MDA-MB468) and Triple Negative/Claudin-Low (MDA-MB231). All cell lines grew in monolayer. From MCF-7, SKBR3 and MDA-MB231 we further generated mammary cancer stem cells (BCSC). We analyzed the mRNA expression of stem cell markers (CD24 and CD44) and immune suppressor ligands (PDL1 and PDL2) by qRT-PCR using TaqMan® Gene Expression Assays (Life Technologies). PUM1 and β-actin were used as reference genes and 184A1 cell line as control sample. Three biological replicates were included and all experiments were done by duplicates. Relative changes in gene expression were calculated as the fold change by the $2^{-\Delta\Delta Ct}$ method. Data analysis was performed by comparing the expression average and standard deviation among all BC cell lines and the results were plotted with GraphPad Prism.

Results: We had a successful generation of BCSC since they showed a $CD44^{high}/CD24^{low}$ mRNA expression profile. Indeed, CD24 mRNA levels decreased significantly in the mammosphere culture. PDL1 and PDL2 mRNA expression was either low or absent in 5 cell lines but high in MDA-MB231. PDL1 expression further decreased in MDA-MB231 and MCF-7 mammospheres but increased in those derived from SKBR-3. In contrast, PDL2 expression did not change significantly.

Conclusions: Our results support the role of PDL1 in the mechanisms of immune control evasion, specifically in aggressive TNBC. The fact that mammosphere cells compared with monolayer cultures underexpresses this ligand further suggests that more differentiated BC cells exploit its expression to subvert T-cell-mediated

immunosurveillance.

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176 SMO mRNA Expression in Breast Cancer: A Clinicopathological Study

Araceli Garcia-Martinez[1], *Marta Garcia-Escolano*[1], *Ariadna Perez-Balaguer*[2], *Fernando Ortiz-Martinez*[1], *Eloy Pomares-Navarro*[3], *Silvia Delgado-Garcia*[4], *Tina Martin*[1], *F Ignacio Aranda*[1], *Gloria Peiro Cabrera*[5]. [1]University General Hospital Alicante, Spain, [2]University General Hospital Alicante and ISABIAL-FISABIO, Alicante, Spain, Alicante, [3]University General Hospital and ISABIAL-FISABIO; Alicante Spain, Alicante, [4]University General Hospital Alicante and ISABIAL-FISABIO, Alicante, [5]University General Hospital Alicante, Spain, Alicante

Background: The Hedgehog (Hh) signaling network regulates organogenesis, cell fate, proliferation, survival and stem cell self-renewal in many mammalian tissues. SMO is a G protein-coupled receptor-like molecule that is essential for the actions of the Hh family of secreted proteins. It is known that SMO-dependent (type II) non-canonical Hh signaling promotes endothelial cell tubulogenesis, fibroblast and non-mammary cancer cell migration. Nonetheless, currently, the existence and potential role of this signaling mechanism in breast carcinogenesis (BC) has not been extensively investigated.

Design: We selected a non-consecutive series of 193 patients with BC: Luminal A (51; 25.9%), Luminal B/HER2- (32; 16.2%), Luminal B/HER2+ (31; 15.7%), HER2-enriched (22; 11.2%) and Triple-Negative/Basal-Like (TN/BL) (57; 28.9%). We analyzed SMO mRNA expression by qRT-PCR using TaqMan® Gene Expression Assays. Relative changes in gene expression were calculated as the fold-change by the 2^{-Ct} method. *PUM1* was the reference gene and normal breast tissue was used as control sample. The results were correlated with clinicopathological factors and prognosis.

Results: The median (P_{25}-P_{75}) value for *SMO* mRNA fold change in tumors was 0.3 (0-0.67). Increased *SMO* expression was seen in tumors with non-vascular invasion (p=0.003), negative lymph-nodes (p=0.024) or TN/BL (p=0.099) (Mann-Whitney U and Kruskal-Wallis tests). Univariate analysis showed that tumour grade 3, lymph-vascular invasion and positive lymph-node status correlated with shorter DFS (all $p \leq 0.050$) and OS (all $p \leq 0.047$). Further, DFS and OS were also shorter in patients whose tumors expressed *SMO* (p=0.068 and p=0.023; respectively) especially among those with HER2 phenotype (p= 0.006 and p= 0.003, respectively). Multivariate analysis showed that only positive lymph-node status (HR=1.796, 95%CI=1.105-2.900, p=0.018) and high SMO (HR=2.521, 95%CI=1.136-5.594, p=0.023) remained as independent poor prognostic factors for DFS and for OS (lymph-node status: HR=2.090, 95%CI=1.252-3.487, p=0.005; *SMO*: HR=2.631, 95%CI=1.187-5.831, p=0.017) (Kaplan-Meier, log rank test and Cox model).

Conclusions: Our results support the involvement of SMO in BC with good histological factors. Furthermore, SMO levels stratified patients at risk of recurrence and/or death in HER2 phenotype and it showed an independent prognostic value. Therefore, SMO is a potential biomarker in BC.

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177 Biomarkers of Targeted Treatment in Neuroendocrine Breast Carcinomas

Zoran Gatalica[1], *Juan Palazzo*[2], *Souzan Sanati*[3], *Elena T Florento*[4], *Joanne Xiu*[5], *Jeffrey Swensen*[5], *Semir Vranic*[6]. [1]Caris Life Sciences, Phoenix, AZ, [2]Thomas Jefferson Medical College, Philadelphia, PA, [3]Washington University, Saint Louis, MO, [4]Caris Life Sciences, Phoenix, AZ, [5]Caris Life Sciences, [6]College of Medicine, Qatar University, Doha, Qatar

Disclosures: Jeffrey Swensen: *Employee*, Caris Life Sciences

Background: Neuroendocrine carcinoma of the breast (NCB) is a rare primary breast malignancy without currently recognized targeted treatment options. Recently described successful targeting of DLL3 with rovalpituzumab tesirine in recurrent small cell lung cancer (Rudin et al. 2017) led us to investigate expression of this and other biomarkers of targeted therapy in NCB.

Design: Formalin-fixed paraffin-embedded tissue samples from 21 patients with NCBs were analyzed for Delta like protein 3 (DLL3), Folate receptor 1 (FOLR1), trimethylation of Lys-36 of histone 3 (H3K36me3) and neurotrophic receptor kinases 1-3 (NTRK) using immunohistochemistry. Somatic genomic alterations (gene fusions, mutations and copy number variations) were explored using next-generation sequencing assays (NGS). O-6-Methylguanine-DNA Methyltransferase (MGMT) protein expression was evaluated using IHC and promoter gene methylation by pyrosequencing.

Results: DLL3 overexpression was observed in 11/19 cases: Two cases had high (>50% of cells) and 9 cases low (1-50%) expression; FOLR1 expression was detected in 6/19 cases with H-score≥20 in 4 cases. A complete loss of H3K36me3 was seen in 4/15 cases (one case harbored *SETD2* gene mutation) while none of the cases (0/20) had any NTRK expression. NGS assays revealed complex genetic alterations among which gene amplification in the fibroblast growth factors pathway (*FGFR1, FGF3, FGF4*) and cyclin D1 (*CCND1*) were more common. Pathogenic mutations were rare (*TP53, SETD2, PIK3CA, IDH1, Rb1).* No gene fusions were detected. No hypermethylation of MGMT promoter was detected. Total mutational load was low (average of 5/Mb). No microsatellite instability was detected.

Conclusions: This study for the first time showed potential for targeted therapy in NCB. Predictive expression levels of DLL3, FOLR1 and H3K36Me3 were detected in different subpopulations of patients. Gene alterations seen in common breast carcinomas including cell cycle control and FGFR pathway were also found in NCB, suggesting relevance of cell cycle inhibitors and FGFR inhibitors in isolated cases of this rare cancer. Additional biomarker support for the use of histone deacetylase inhibitors (HDAC) may be explored in selected NCBs.

178 HMGA2 Rearrangement in Breast Adenomyoepitheliomas Lacking HRAS and PI3K Pathway-Related Gene Mutations

Felipe Geyer[1], *Anqi Li*[2], *Fresia Pareja*[3], *Kathleen A Burke*[4], *Marcia Edelweiss*[5], *Hannah Y Wen*[6], *Achim Jungbluth*[5], *Zsuzsanna Varga*[7], *Brian P Rubin*[8], *Emad Rakha*[9], *Ian Ellis*[10], *Britta Weigelt*[6], *Jorge Reis-Filho*[11]. [1]Memorial Sloan-Kettering Cancer Center, New York, NY, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]MSKCC, New York, NY, [4]MSKCC, [5]MSKCC, New York, NY, [6]Memorial Sloan Kettering Cancer Center, New York, NY, [7]University Hospital, Zurich, Switzerland, [8]Cleveland Clinic, Cleveland, OH, [9]University of Nottingham, Nottingham, England, [10]City Hospital, NHS Trust, Nottingham, England, [11]Memorial Sloan-Kettering CC, New York, NY

Background: Breast adenomyoepitheliomas (AMEs) are rare lesions with epithelial-myoepithelial differentiation that may occasionally display areas with myxochondroid matrix akin to pleomorphic adenomas (PAs). Whilst a subset of AMEs and salivary gland epithelial-myoepithelial carcinomas are underpinned by hotspot mutations affecting *HRAS* and/or *PIK3CA*, salivary gland PAs harbor rearrangements affecting *HMGA2* or *PLAG1*. We sought to define whether a subset of AMEs would be underpinned by fusion genes, in particular those affecting *HMGA2* or *PLAG1*.

Design: Thirteen AMEs were retrieved from the authors' institutions and subjected to central pathology review. Estrogen receptor (ER) status was assessed by immunohistochemistry following current ASCO/CAP guidelines. Mutations affecting the hotspot loci of *HRAS* Q61 and *PIK3CA* were evaluated by Sanger sequencing. Five cases were subjected to RNA-sequencing for fusion gene discovery using validated bioinformatics algorithms. Twelve cases were subjected to fluorescence *in situ* hybridization (FISH) using split-apart probes targeting *HMGA2* and *PLAG1*.

Results: Seven AMEs were ER-positive, of which 5 were *PIK3CA*-mutant and all *HRAS*-wild-type (WT). Six AMEs were ER-negative, of which 5 were *HRAS*-mutant, 5 *PIK3CA*-mutant, and 4 double-mutant. RNA-sequencing revealed the *HMGA2-WIF1* fusion gene, resulting in a chimeric transcript composed of exons 1-5 of *HMGA2* and exons 3-10 of *WIF1*, in an ER-positive, $HRAS^{WT}$/$PIK3CA^{WT}$AME, which lacked myxochondroid matrix and atypical features (e.g., increased mitotic activity, necrosis and nuclear pleomorphism). FISH validated the *HMGA2* rearrangement, which was found to be present in myoepithelial and epithelial tumor cells. RNA-sequencing identified no additional in-frame fusion gene in the remaining 4 AMEs analyzed, and no additional AME was found to harbor *HMGA2* or *PLAG1* rearrangements by FISH.

Conclusions: Breast AMEs are heterogeneous. Whilst ER-positive AMEs harbor mutations affecting PI3K pathway-related genes, ER-negative AMEs display Q61 *HRAS* hotspot mutations often in conjunction with mutations affecting PI3K pathway-related genes. One AME lacking mutations affecting *HRAS* and PI3K pathway-related genes was found to harbor an *HMGA2-WIF1* fusion gene, despite the histologic features remarkably similar to those of the remaining AMEs, suggesting that a subset of breast AMEs may be related to pleomorphic adenomas.

179 Prognostic Assessment of Stromal and Non-Stromal Tumor-Infiltrating Lymphocytes (TILs) in Triple-Negative Breast Cancer (TNBC)

Toshi Ghosh[1], *Matthew Goetz*[2], *Roberto A Leon-Ferre*[2], *Heshan Liu*[2], *Mei-Yin C Polley*[2], *James Ingle*[3], *Victoria L Cafourek*[2], *David W Hillman*[1], *Jodi Carter*[4], *Daniel Visscher*[4]. [1]Mayo Clinic, Rochester, MN, [2]Mayo Clinic, Rochester, MN, [3]Rochester, MN, [4]Mayo Clinic, Rochester, MN

Background: Most TNBC are high-grade, making it difficult to assess prognosis using histological factors. We and others have demonstrated that stromal TILs (sTILs) are prognostically significant in TNBC. The 2014 International TILs Working Group (WG) and 2017 International Immunooncology Biomarkers WG recommend that evaluations focus

on sTILs. We compared the prognostic utility of sTILs, intratumoral TILs (iTILs), and total lymphoid infiltrate (TLI) in TNBC.

Design: 605 of 9,982 women (median age, 56.3 years) who underwent breast surgery at a single institution between 1985 and 2012 met criteria for TNBC (ER/PR <1%, HER2-negative), as previously reported (Leon-Ferre et al., Breast Cancer Res Treat, 2017). sTILs (per 2014/2017 International WG guidelines), iTILs, and TLI (all lymphoid infiltrate in one representative tumor section, including sTILs, iTILs, and in adjacent normal tissue) were assessed and correlated with growth pattern, fibroblasts, and fibrosis via Spearman, Kruskal-Wallis, and Wilcoxon Rank-Sum analyses. IDFS and OS were assessed via Kaplan-Meier analysis and Cox proportional hazards models.

Results: Subtypes included invasive carcinoma of no specific type (n=503), metaplastic (n=46), apocrine (n=37), and medullary (n=19). Improved IDFS and OS, respectively, were significantly associated with increased sTILs (P<0.0001 and <0.0001), iTILs (P<0.0001 and =0.002), and TLI (P=0.001 and =0.01) on univariate and multivariate analyses. In patients with moderate-to-high TLI, 10-year IDFS and OS were 64.5% (95% CI: 57.0-73.1%) and 73.7% (95% CI: 67.7-80.2%), compared to 47.8% (95% CI: 41.6-54.9%) and 60.4% (95% CI: 54.7-66.7%) in none-to-low TLI. There was a positive correlation between sTILs and iTILs (ρ=0.64), iTILs and TLI (ρ=0.69), and sTILs and TLI (ρ=0.82) (P<0.0001). 2/19 patients with classic medullary carcinoma died of disease. Circumscribed pattern correlated with higher sTILs, iTILs, and TLI than infiltrative (P<0.0001). Fibroblasts and fibrosis, respectively, negatively correlated with sTILs (ρ=-0.34, -0.45), iTILs (ρ=-0.39, -0.26), and TLI (ρ=-0.30, -0.33) (P<0.0001).

Table 1. Distribution of stromal TILs, intratumoral TILs, and total lymphoid infiltrate in triple-negative breast cancer.

Triple-Negative Breast Cancer (n=605)	
Stromal TILs	
0-10%	202 (33.4%)
11-20%	130 (21.5%)
21-40%	144 (23.8%)
>40%	122 (20.2%)
Not available	6 (1.0%)
Median % (range)	20% (0-90%)
Intratumoral TILs	
0%	242 (40.1%)
0-5%	151 (25.0%)
6-10%	73 (12.1%)
11-20%	79 (13.1%)
21-30%	31 (5.1%)
>30%	11 (1.8%)
Not available	17 (2.8%)
Median % (range)	2.5% (0-60%)
Total Lymphoid Infiltrate	
0 (none)	98 (16.2%)
1 (low)	243 (41.0%)
2 (moderate)	153 (25.3%)
3 (high)	86 (14.2%)
Not available	20 (3.3%)

Figure 1. **Kaplan-Meier curves of invasive disease-free survival by stromal TILs, intratumoral TILs, and total lymphoid infiltrate.**

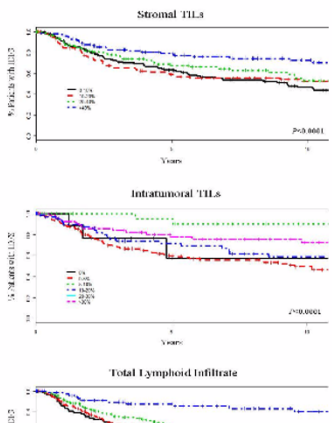
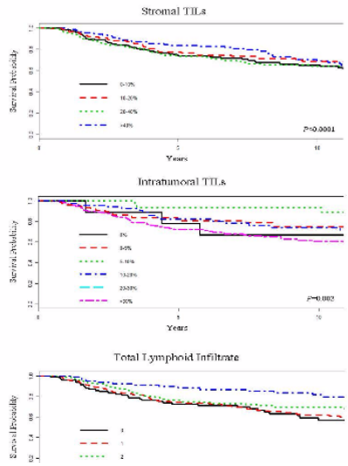


Figure 2. **Kaplan-Meier curves of overall survival by stromal TILs, intratumoral TILs, and total lymphoid infiltrate.**



Conclusions: TNBC with high TILs constitutes a biologically and clinically distinct disease subset. Circumscribed growth correlated with high TILs, while fibroblasts and fibrosis negatively correlated with TILs. sTILs, iTILs, and TLI positively correlated and predicted favorable outcomes. This suggests these TILs may be functionally similar and that TILS may be adequately evaluated via any of these three measures.

180 Methylation Analysis of Non-Luminal Breast Carcinoma and Breast Cancer Cell Lines

Micaela Giani Alonso[1], *Araceli Garcia-Martinez*[2], *Marta Garcia-Escolano*[2], *Estela Costa*[2], *Fernando Ortiz-Martinez*[2], *Raquel Linares Fernández*[3], *Jose Ponce*[2], *Silvia Delgado-García*[4], *Hortensia Ballester*[2], *Tina Martin*[2], *F Ignacio Aranda*[2], *Gloria Peiro Cabrera*[5]. [1]University General Hospital and ISABIAL-FISABIO, Alicante, Alicante, [2]University General Hospital Alicante, Spain, [3]University General Hospital ISABIAL-Alicante, Alicante, [4]University General Hospital Alicante and ISABIAL-FISABIO, Alicante, [5]University General Hospital Alicante, Spain

Background: DNA methylation has been frequently observed in a variety of human cancers. Prior experimental studies support the contribution of hypermethylation to neoplastic transformation through generation of chromosomal instability, reactivation of transposable elements and loss of genomic imprinting. In breast carcinoma (BC) of non-luminal type, promoter hypermethylation as a mechanism responsible for *ERS1* gene silencing has not been extensively evaluated.

Design: We included 110 non-consecutive invasive BCs with negative hormonal receptor status determined by immunohistochemistry: HER2-enriched (25/110, 23%) and Triple-negative/Basal-like -TN/BL- (85/110, 77%) phenotypes. Using a commercial kit, we analyzed the methylation profile of 26 cancer related gene regions by Methylation-Specific Multiplex Ligation dependent Probe Amplification technique (MS-MLPA). The methylation results of the genes were correlated, as well as with clinicopathological factors (age, histological grade, lymph-vascular invasion, necrosis, lymph-node status and immunophenotype) and patients' outcome. The results were corroborated in BC cell lines: SKBR3 (HER2-enriched), MDA-MB231 and MDA-MB468 (both TN/BL).

Results: The most frequently methylated genes were *APC* (40.2 %), *CDKN2B* (35.6 %) and *RASSF1* (35.6%). However, *ESR1* silencing was found in only 9.4% of the samples. Interestingly, several co-methylations were found: *APC* and *RASSF1* (p<0.000), *APC* and *CD44* (p=0.003), *APC* and *CDKN2B* (p=0.003); and finally, *BRCA1* and *KLLN* (p<0.000). Methylation of *APC* was related to HER2-enriched phenotype (p=0.05), whereas methylation of *CDKN2B* was associated with younger age (p=0.03). Nevertheless, no associations were found between gene methylation status and prognosis (Kaplan-Meier method; log-rank test; all p=ns). RASSF1 appeared also methylated in the three cell lines. Besides this, each cell line showed its own methylation profile, being MDA-MB468 with the most methylated genes (*CDH13, TIMP3, RARB, ESR1, TP73, CADM1 and GSTP1*).

Conclusions: Our data showed that *ERS1* methylation is responsible for ER-negative status in a subset (9.4%) of BC of non-luminal type determined by immunohistochemical methods. Furthermore, hypermethylation of *APC, CDKN2B* and *RASSF1* is a relatively frequent event in BC, and specifically APC is detected in HER2-enriched subtype. However, none had prognostic impact.

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181 Molecular Characterization of Metaplastic Breast Carcinoma via Next Generation Sequencing

Gabriel Giannini[1], *Mark Ewalt*[2], *Jing Zhai*[3], *Lily Lai*[4]. [1]Cedars Sinai Medical Center, [2]Cedars-Sinai Medical Center, Los Angeles, CA, [3]Cedars Sinai Medical Center, Los Angeles, CA, [4]City of Hope National Medical Center, Duarte, CA

Background: Metaplastic breast carcinoma (MBC) is a rare and heterogenous subtype of breast cancer with higher clinical stage and a poor clinical outcome compared to usual infiltrating ductal carcinoma. The standard chemotherapeutic regimens do not appear to be effective treatments for MBC. The management of patients with MBC presents a challenge to clinicians. The purpose of our study is to identify molecular alterations in MBC using next generation sequencing (NGS), which may aid chemotherapeutic reagent selection and be promising in identifying targeted therapy.

Design: A cohort of 18 patients with MBC were identified in the department database. Microdissection was performed on formalin fixed and paraffin embedded tumor blocks, and genomic DNA was extracted. The Ion AmpliSeq cancer hotspot mutation panel Version 2 kit by Life Technologies was utilized. It targets ~2800 hotspot mutations in 50 oncogenes and tumor suppressor genes frequently mutated in human cancers.

Results: A total of 23 genetic alterations were identified in 15 of 18 patients (83.3%). Eleven genetic alterations involved in PI3K signaling pathway were identified in 9 of 18 patients (50.0%), including 7 PIK3CA mutations, 3 PTEN genetic alterations, and 1 AKT1 mutation. Ten of 18 patients (55.6%) each harbored one P53 genetic alteration. Additional genetic alterations identified were one HRAS mutation and one ATM mutation.

Conclusions: NGS analysis demonstrated that PI3K pathway related genetic alterations are present in a high percentage of MBC. This suggests that therapy targeting the PI3K/mTOR pathway may be promising for MBC treatment.

182 Identification of HER2 Immunohistochemistry-Negative, FISH-Amplified Breast Cancers and their Response to Anti-HER2 Neoadjuvant Chemotherapy

I-sanna R Gibbons-Fideler[1], *Hiro Nitta*[2], *Peter Banks*[3], *Gary Tozbikian*[4], *Anil Parwani*[5], *Zaibo Li*[6]. [1]The Ohio State University, Columbus, OH, [2]Ventana Medical Systems, Inc., Tucson, AZ, [3]Charlotte, NC, [4]The Ohio State University, Columbus, OH, [5]The Ohio State University, Columbus, OH, [6]Ohio State University Wexner Medical Center, Columbus, OH

Background: Either immunohistochemistry (IHC) or in situ hybridization (ISH) can be used to determine HER2 status in breast carcinoma (BC). Most institutions use IHC primarily and then reflex to ISH for equivocal cases (IHC 2+). Recent studies have reported BCs with HER2 IHC-negative (IHC-) and ISH-amplified (ISH+) results. In our institution, both IHC and fluorescence ISH (FISH) are simultaneously performed for initial assessment on core biopsies of invasive BCs. We aimed to investigate the frequency of HER2 IHC-/ISH+ BCs and their response to anti-HER2 neoadjuvant chemotherapy (NAC).

Design: The HER2 statuses of 1,107 consecutive invasive BCs during a 3-year period (November, 2013 – December, 2016) were assessed by both IHC and FISH and the results were interpreted using the new 2013 criteria. In order to confirm the HER2 IHC and FISH results, gene protein assay (GPA) combining HER2 IHC and dual ISH was also performed on original blocks and representative images were shown in Figure.

Results: Seventeen BCs with HER2 IHC-/ISH+ results were identified (1.5%, 17/1,107). The detailed clinicopathologic characteristics were summarized in Table. GPA was performed on 13 of 17 cases and confirmed the original HER2 IHC and ISH results. Four cases had HER2 IHC on resections with 3 as 1+ and 1 as 2+. Two cases had HER2 FISH on resections with 1 as amplified and the other as non-amplified.

Eight patients had anti-HER2 NAC and 3 had pathologic complete response and 5 had residual tumors. Residual cancer burden was analyzed on all 5 cases, with 2 as RCB-I and 3 as RCB-II. (Table 1)

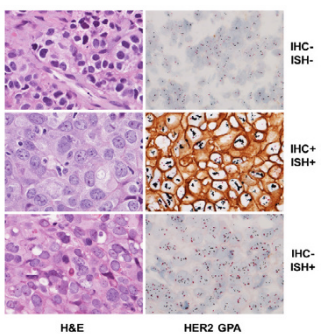


Table: Clinicopathologic characteristics of seventeen invasive breast carcinomas with HER2 IHC-/FISH+ results.

		Average/case #	Range/%
Age (years)		57.1	30-95
Tumor type	Ductal	17	100%
Nottingham grade	1	1	5.9%
	2	5	29.4%
	3	11	64.7%
Nuclear grade	2	4	23.5%
	3	13	76.5%
ER-positive		6	35.3%
PR-positive		6	35.3%
HER2 IHC	1+	17	100%
HER2 FISH	HER2 signals/cell	6.86	3.60-19.30
	HER2/Ch17 ratio	2.45	1.29-5.57
Lymph node status	Negative	6	35.3%
	Positive	8	47.1%
	unknown	3	17.6%
Neoadjuvant chemotherapy	Cases with pCR	3	37.5%
	Cases with residual tumor	5	62.5%
	RCB-I	2	25%
	RCB-II	3	37.5%

Note: Six cases with HER2/Ch 17 ratio < 2, but HER2 signals/cells > 6.
Abbreviations: ER: estrogen receptor; PR: progesterone receptor; IHC: immunohistochemistry; pCR: pathologic complete response; RCB: residual cancer burden.

Conclusions: A small cohort of patients (1.5%) showed discordant HER2 IHC and ISH results (IHC-/ISH+) and might lose the opportunity of potentially beneficial anti-HER2 targeted therapy if reflex HER2 testing strategy based on IHC result was used.

183 Role of Differential Subcellular Localization of Protein ZNF217 and CtBP1 in Breast Cancer Prognosis and outcome

Sara M Gil[1], *Sam Park*[2], *Erick Mahoney*[2], *Kevin Gardner*[2]. [1]National Institutes of Health, Silver Spring, MD, [2]National Institutes of Health

Background: The Zinc finger protein ZNF217 is encoded by the 20q13 chromosomal region, frequently amplified in different tumors Including breast cancer. The C-terminal Binding Protein 1 (CtBP1) is a member of a dimeric nuclear transcriptional repressor family that has been shown to interact with a wide range of proteins, including ZNF217and act as a metabolic sensor through its stabilization by low NAD+/NADH levels. Within the cell, ZNF217 and CtBP1 have multiple distinct functions in the nucleus and the cytoplasm. Using immunohistochemistry (IHC), this project will focus on the correlated roles and associations of ZNF217 and CtBP1 protein levels in breast cancer, and how their differential subcellular localization may predict tumor status and patient outcome.

Design: Data from a cross-sectional cohort of 558 breast cancer patient samples with demographic data and clinical-pathological information were extracted from the medical records. The levels of Nuclear and Cytoplasmic protein expression were measured by IHC and digital quantification through Aperio ScanScope CS slide Scanner, also RNA sequence data for a subset of 140 patients was made available for analysis. A statistical and computational analysis was performed to determine the significant prognostic associations between the expression of ZNF217 and CtBP1 within tumor subtype, stage, and grade.

Results: Our results indicate that ZNF217 and CtBP1 Nuclear expression is strongly associated with ER+ tumors while high cytoplasmic CtBP1 is not. In contrast nuclear ZNF217 is associated with ER+ tumors and a lower proliferation index while high cytoplasmic ZNF217 correlates more strongly with ER- status and high proliferation index. Notably low cytoplasmic CtBP1 is associated with significantly poorer survival in ER+ patients while high cytoplasmic ZNF217 predicts favorable survival. RNA levels of ZNF217 and CtBP1 do not show similar correlation with subtype or survival. These studies demonstrate that differential subcellular localization of gene regulatory factors have different prognostic results.

Conclusions: These data suggest differential functional interactions between CtBP1 and ZNF217 depending on subcellular localization. We discuss the discordance between ZNF217 and CtBP1 mRNA with protein levels in breast cancer.

184 Impact of Estrogen Receptor Variants on Allred Score and Breast Cancer Outcomes

Yesim Gokmen-Polar[1], *Yaseswini Neelamraju*[2], *Steven A Buechler*[3], *Sunil Badve*[4]. [1]Indiana University School of Medicine, Indianapolis, IN, [2]Univ of Virginia, Charlottesville, VA, [3]Notre Dame University, Notre Dame, IN, [4]Indiana Univ/Medicine, Indianapolis, IN

Background: Estrogen receptor (ER) is a strong predictor to response to endocrine therapy. Immuno-histochemical (IHC) staining of ER protein expression is the gold standard for the selection of patients who will respond to endocrine therapy. With the advent of mRNA expression based techniques, ER (*ESR1*) mRNA expression is increasingly used as a predictor of response to endocrine therapy.

Alternative splicing of ER has been described, but its impact on protein expression and outcomes is not known.

Design: After appropriate permissions, the Cancer Genome Atlas (TCGA) BRCA cohort gene expression and survival data was downloaded from the GDC website. Expression of transcripts of *ESR1* gene (15 transcripts as per Ensembl GRCh38.p10) was correlated with protein expression by immunohistochemistry (Allred Score). Expression of protein and transcripts was correlated with relapse-free survival (RFS).

Results: Expression of 10 *ESR1* transcripts was detectable in TCGA BRCA cases. The median expression of these was <1 TPM (Transcripts Per Kilobase Million) in ER-negative cases. In ER-positive (ERpos) cases, 7 out of 10 *ESR1* variants were expressed at very low levels (TPM <1). The expression of the remaining three transcripts (including the primary transcript ESR1-01) ranged from 0-200 TPMs. ESR1-01 levels were associated with Allred Score (Pearson's r =0.43), with increasing levels being associated with higher score. However, the expression of the additional transcripts was only weakly associated with expression of the Allred score (Pearson's r =0.48 and 0.44) and with ESR1-01 levels (Pearson's r =0.37 and 0.63). The expression levels of the ESR1-01 and ESR1-02 were not associated with prognosis (log-rank P value 0.81 and 0.07 respectively). In contrast, expressions of the other ESR1-03 was inversely associated with RFS (log-rank P value 0.0009).

Conclusions: Analysis of the TCGA BRCA cohort documents the importance of complexity of the gene expression. This further emphasizes the need for transcript specific analysis in determination of outcomes of ER+ patients.

185 Utility of Screening for Neuroendocrine Differentiation and Its Relationship to FGFR1 Amplification in ER+ Breast Cancer

Paula Gonzalez Ericsson[1], *Jennifer Giltnane*[2], *Maria G Kuba*[3], *Monica V Estrada*[4], *Ingrid A Mayer*[5], *Ingrid Meszoely*[5], *Justin Balko*[5], *Melinda E Sanders*[1], *Katherine E Hutchinson*[6]. [1]Vanderbilt University Medical Center, Nashville, TN, [2]Genentech, [3]Brigham and Women's Hospital, [4]University of California San Diego, [5]Vanderbilt University Medical Center, [6]Genentech, Inc., South San Francisco, CA

Background: Significance of neuroendocrine differentiation (NED) in breast cancer (BC) is controversial. Well-differentiated neuroendocrine tumors and small cell carcinomas of the breast are rare; however, NED by immunohistochemistry (IHC) may be seen in up to 30% of BC. Literature suggests NED (>50% of tumor) associates with poor prognosis. tNGS of a few cases reveals recurrent alterations including *FGFR1* amplification (amp). *FGFR1*amp occurs in approximately 15% of BC and associates with poor prognosis and resistance to endocrine and CDK4/6 inhibitor therapies. We investigated the relationship of NED and *FGFR1*amp in ER+ BC.

Design: We screened ER+ BC TMAs for evidence of NED by IHC [synaptophysin (syn) +/- chromogranin A (chA)] and for *FGFR1*amp by fluorescence in situ hybridization, including 85 tumors from patients who received presurgical letrozole. Tumors were grouped by morphology for correlation with molecular findings: neuroendocrine/solid (NES) growth pattern, invasive micropapillary features (InvMPF), mucin production, special type and no special type. Fisher's exact and Student's t-tests were used for comparisons. Survival analyses utilized the Kaplan-Meier method. All tests were 2-tailed with p values <0.05 considered significant.

Results: 349 primary ER+ BC were studied. Thirty (8.6%) showed NED in >1% cells (27 syn+ [7.7%], 12 chA+ [3.4%] and 8 both [2.3%]). Ten tumors (2.9%) expressed one or both markers in >50% of cells. *FGFR1*amp was present in 23 tumors (6.6%), including six (26%) with NED. NED associated with NES growth pattern (p=0.0001), *FGFR1*amp (p=0.009), and recurrence (p=0.02). *FGFR1*amp associated with NES growth pattern (p=0.01), InvMPF (p=0.05) and intermediate-high grade (p=0.005). Tumors with *FGFR1*amp also had higher Ki67 index (≥14% vs. <14%) than non-amplified tumors (p=0.008). Follow-up information was available for 240 patients including 85 who received presurgical letrozole. Among patients whose tumors demonstrated NED, mean disease-free survival and disease-specific survival were 81 and 87 months vs. 106 (p=0.007) and 111 months (p=0.08) for those without NED. Five of 6 *FGFR1*amp tumors (p=0.002) and 2 of 5 tumors with NED were among 24 tumors categorized as resistant based on lack of ΔKi67 per IMPACT criteria.

Conclusions: BCs with morphological or IHC evidence of NED are associated with a significantly worse prognosis and *FGFR1*amp. In specific subsets of BC, NED may identify tumors harboring *FGFR1*amp and resistant to endocrine and CDK4/6 inhibitor therapy.

186 Oncotype DX Recurrence Score in Multifocal/ Multicentric Ipsilateral Invasive Breast Carcinomas

Anne Grabenstetter[1], *Edi Brogi*[2], *Joanne F Chou*[3], *Monica Morrow*[3], *Maura Dickler*[3], *Larry Norton*[3], *Hannah Y Wen*[2]. [1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]Memorial Sloan Kettering Cancer Center

Disclosures: Monica Morrow: *Consultant*, Genomic Health

Background: The 21-gene recurrence score assay (Oncotype DX™) predicts risk of distant recurrence and benefit of chemotherapy in patients with early stage, ER+, HER2- breast cancer (BC). The recurrence score (RS) stratifies patients into 3 risk categories: low (RS <18), intermediate (RS 18-30) and high (RS ≥31). This study examined the concordance of the RS in multiple synchronous ipsilateral BC with similar histology.

Design: We retrospectively reviewed our database and identified patients with multiple synchronous ipsilateral BC with available RS treated at our institution from 1/2014 to 4/2017. BCs with different morphology were excluded. RS results were divided into 3 groups: 1) RS falls in same risk category, 2) RS in different risk categories but within 2-unit difference (e.g. RS 17 and RS 19), and 3) RS in different risk categories and a change of >2 units. Groups 1 and 2 were defined as concordant (no significant clinical impact) and group 3 was discordant (variation affects management). Two pathologists reviewed the H&E slides for group 2 and 3 cases.

Results: A total of 40 patients with multiple foci of BC and available RS met the inclusion criteria. The patient median age was 52 years (range 32-73). Tumor types include ductal (n=25), lobular (n=13) and mixed (n=2). The median tumor size was 1.3 cm (range 0.5-4.4 cm). Most (85%, 34/40) patients had two tumors tested, 13% (5/40) had 3 and 2% (1/40) had 4. RS was concordant in 36 (90%) cases, including 34 (85%) in group 1, and 2 (5%) in group 2. The median difference of RS in group 1 was 2 (range 0-11). One patient in low risk category had RS scores of 0 and 11. The median difference of group 2 was 1.5 (range 1-2). Four (10%) cases were discordant (group 3); median RS difference was 9 (range 4-10). Table 1 summarizes the group 3 cases. Of these, 3 (75%, 3/4) had biopsy cavity changes (BXC) adjacent to the BC focus associated with higher RS. In 1 (25%) case no BXC were identified. In this case, PR expression by immunohistochemistry was at a slightly lower level (40% vs 70%) in the focus with higher RS.

Case	Tumor type	MBR Grade	Tumor size (cm)	Recurrence Score	Risk Classification
1	Lobular with pleomorphic features	2	1.8	15	Low
			3.0	19*	Intermediate
2	Ductal, NOS	1	0.6	11	Low
			0.8	20*	Intermediate
3	Mixed	2	1.0	12	Low
			1.2	19*	Intermediate
			0.9	21*	Intermediate
4	Ductal with micropapillary features	2	1.0	13	Low
			0.8	23	Intermediate

Table 1: Summary of Group 3 cases. *Focus with adjacent BXC

Conclusions: Our data shows that multifocal ipsilateral BCs with similar morphology have concordant RS in 90% of cases. Biopsy changes contributed to increased RS in 3 of 4 discordant cases, emphasizing the importance of avoiding the biopsy site in samples selected for testing. Our results suggest that analysis of only one focus of morphologically similar multifocal ipsilateral BC provides accurate prognostic information.

187 The 2017 Guidelines will Decrease HER2 Positivity Rates in Breast Cancer

H. Evin Gulbahce, **Background:** A focused update of American Society of Clinical Oncology (ASCO) and College of American Pathologist's (CAP) guideline recommendations for HER2 testing in breast cancer was published in 5/2017 for public comment. At the time of submission of this abstract, the new guidelines were not published yet. From the following in situ hybridization (ISH) scenarios: 1) HER2/CEP17: ≥ 2.0, HER2 /cell <4.0; 2) HER2/CEP17: <2.0, HER2 / cell ≥6.0; 3) HER2/CEP17 <2.0, HER2 /cell ≥4.0 - <6.0, the first two groups are currently reported as positive, the third as equivocal. It is recommended that immunohistochemistry (IHC) performed by the same lab for all these groups. If the IHC testing is 3+ or 0/1+ the tests reported as positive or negative respectively. If the reflex IHC is 2+, a recount of the original ISH area with IHC 2+ is recommended. The purpose of this study is to review the results of targeted FISH to predict the effects of proposed guidelines in a national reference lab.

Design: HER2 FISH tests performed between 4/2015-5/2017 are included. Our lab offers HER2 testing by IHC (HercepTest™ Dako) and dual probe FISH (Dako IQ). Equivocal (2+) cases showing ≥10% weak or moderate circumferential staining or intense but <10% circumferential staining is circled and reflexed to HER2 FISH with preferential counting in the circled areas. Equivocal FISH cases are re-tested with

the alternate RAI1 probe (Agilent Technologies). FISH scoring is done manually by 2 people following 2013 ASCO/CAP guidelines with at least 20 cells counted in amplified and non-amplified and 40 cells counted in equivocal cases.

Results: 2,460 HER2 FISH test requests were received during the study period. 7 and 13 cases failed initial and repeat testing of equivocal cases with reflex probe respectively. 389 (16.2%) cases were amplified, 1686 (68.7%) non-amplified, and 369 (15.0%) were equivocal with FDA approved probe set. 116 (32.6%) of equivocal cases re-tested with alternate probe were amplified increasing overall amplification rate to 21%. The table below shows cases that fall under 3 uncommon categories that are proposed for changes with 2017 guidelines.

HER2/ CEP17 Ratio	Aver-age HER2/ Cell	Number of Cases with IHC (Total Number)	IHC n (result)	Results per 2013 ASCO/ CAP before Reflex Probe	Results per 2013 ASCO/CAP after Reflex Probe	Results per Proposed Draft Guidelines
≥ 2	< 4	8 (28)	7 (2+) 1 (1+)	POSITIVE	-	NEGATIVE
< 2	≥ 6	9 (31)	9 (2+)	POSITIVE	-	NEGATIVE
< 2	≥ 4-6	98 (372)	96 (2+) 1 (3+) 1 (0)	EQUIVOCAL	32/98 (32.7%) POSITIVE	1/98 (1%) POSITIVE

Conclusions: In our lab where all FISH tests with prior IHC are counted on targeted areas, all cases within the first two groups will be reclassified as negative. The use of reflex probe in equivocal cases is not recommended in the new recommendations further decreasing positivity rates. A 5% decrease in HER2 FISH positivity rates is expected following 2017 recommendations.

188 PD-L1 Expression (clone 22C3) and Tumor Infiltrating Lymphocytes in Primary Invasive Breast Cancer

Hua Guo[1], *Qingqing Ding*[2], *Yun Gong*[3], *Michael Z Gilcrease*[3], *Min Zhao*[4], *Jun Zhao*[5], *Hui Chen*[6], *Stacy Moulder*[2], *Wei-Lien Billy Wang*[7], *Lei Huo*[2]. [1]MD Anderson Cancer Center, Houston, TX, [2]UT MD Anderson Cancer Center, [3]UT-MD Anderson Cancer Center, Houston, TX, [4]MD Anderson Cancer Center, [5]M D Anderson Cancer Center, Houston, TX, [6]UT MD Anderson Cancer Center, Houston, TX, [7]UT MD Anderson Cancer Ctr, Houston, TX

Background: Data on PD-L1 expression in breast cancer (BC) by IHC staining with the FDA approved clone 22C3 (Dako) are extremely scant. Tumor infiltrating lymphocytes (TILs) have been associated with response to PD-1/PD-L1 inhibitors. In this study, we examine the expression of PD-L1 using clone 22C3 and TILs in BC.

Design: PD-L1 staining was performed on tissue microarrays constructed from primary invasive breast cancer resection specimens of 481 patients diagnosed between 2004 and 2016 and treated in our institution. All patients received standard therapy without PD-1/PD-L1 inhibitors. Stromal TILs (sTILs) was evaluated on corresponding whole slide sections according to the International Working Group guidelines. For PD-L1, membranous staining of ≥1% was considered positive. For sTILs, ≥5%, ≥10% and ≥20% were used as cutoffs. Statistical analysis was performed to assess the association of PD-L1 and sTILs with clinicopathological features and clinical outcome in the entire cohort, pretreatment and post neoadjuvant chemotherapy (NACT) specimen cohorts.

Results: Positive PD-L1 staining was detected in 10.4% (50/481) of tumors and was highest in the triple-negative subtype (17/105; 16.2%). As shown in Table 1, positive PD-L1 was associated with higher histologic grade, higher sTILs, ER(-), PR(-) and triple-negative status. Regardless of cutoff selection, higher sTILs were associated with younger age, ductal subtype, higher histologic grade, lower pT stage, ER(-), PR(-), HER2(+) and triple-negative status (data with 10% cutoff are shown in Table 1). When positive PD-L1 and higher sTILs were combined, histologic grade, ER, PR and triple-negative status remained significant features. When the cohort was divided into pretreatment and post NACT specimens, ER and triple-negative status were not associated with PD-L1 in the pretreatment cohort (Table 1). Follow-up time ranged from 0.4 to 124.3 months (median: 23.0 m). Positive PD-L1 and combined positive PD-L1/high sTILs were associated with worse disease specific survival (DSS) in the pretreatment cohort (P=0.01; P=0.033), and not with overall survival, disease-free survival and distant disease-free survival in any cohort. sTILs were not associated with survival in any cohort.

Table 1. Association of PD-L1 (clone 22C3) and sTILs with clinicopathological features

Clinicopathologic features	P-value		
	PD-L1 (pos vs neg)	sTILs (high vs low)	PD-L1/ sTILs (pos/ high vs others)
Age [N=481 (< 50 yrs: 146; ≥ 50 yrs: 335)]	1.000	0.020*	0.186
Neoadjuvant chemotherapy [N=481 (Yes: 147; No: 334)]	0.519	0.438	0.573
Histology subtype [N=479 (IDC: 408; ILC: 53; Mixed IDC/ILC: 13; Metaplastic carcinoma: 5)]	0.706	<0.001*	0.295
Histologic Grade [N=479 (G1: 72; G2: 219; G3: 188)]	<0.001*#	<0.001*#	<0.001*#
sTIL [N=475 (<5%: 238; 5-9%: 111; 10-19%: 46; ≥20%: 80)]	<0.001*#	N/A	N/A
ER [N=481 (Neg: 112: Pos: 369)]	0.002#	<0.001*#	0.003*#
PR [N=480 (Neg: 171; Pos: 309)]	<0.001*#	<0.001*#	<0.001*#
HER2 [N=481 (Neg: 438; Pos: 43)]	0.793	0.040*	0.544
Receptor subtypes [N=481 (ER+/HER2-: 333; HER+: 43; TN: 105)]	0.072#	<0.001*#	0.012#
Triple Negative [N=481 (Yes: 105; No: 376)]	0.045#	<0.001*#	0.009#
pT stage [N=481 (pT1: 250; pT2: 158; pT3: 57; pT4: 16)]	0.544	0.016#	0.324
pN stage [N=480 (pN0: 275; pN1: 133; pN2: 36; pN3: 36)]	0.600	0.360	0.381
pM stage [N=481 (pM0: 466; pM1: 15)]	1.000	0.373	0.613

P-values of <0.05 were considered statistically significant.

The P-values shown are for the entire cohort. The significant ones are in bold. *P-value was significant in the pretreatment specimen cohort; #P-value was significant in the post-neoadjuvant chemotherapy specimen cohort.

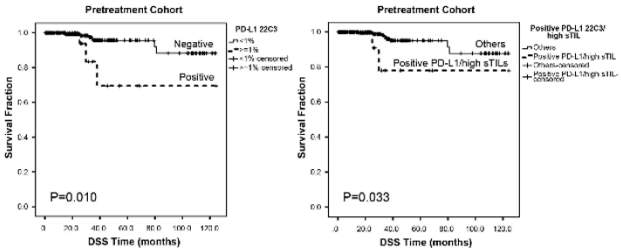


Figure 1. Kaplan-Meier curves of PD-L1 (22C3), sTILs and DSS.

Conclusions: PD-L1 (clone 22C3) was expressed in 10.4% of invasive BCs not treated with PD-1/PD-L1 inhibitors. Its expression was associated with sTILs, histologic grade and ER/PR status. PD-L1 and combined positive PD-L1/high sTILs were associated with DSS in the pretreatment cohort.

189 PD-L1 Expression (clone 28-8) and Tumor Infiltrating Lymphocytes in Primary Invasive Breast Cancer

Hua Guo[1], *Qingqing Ding*[2], *Yun Gong*[3], *Michael Z Gilcrease*[3], *Min Zhao*[4], *jun zhao*[5], *Hui Chen*[6], *Stacy Moulder*[2], *Wei-Lien Billy Wang*[7], *Lei Huo*[2]. [1]MD Anderson Cancer Center, Houston, TX, [2]UT MD Anderson Cancer Center, [3]UT-MD Anderson Cancer Center, Houston, TX, [4]MD Anderson Cancer Center, [5]M D Anderson Cancer Center, Houston, TX, [6]UT MD Anderson Cancer Center, Houston, TX, [7]UT MD Anderson Cancer Ctr, Houston, TX

Background: Data on PD-L1 expression in breast cancer (BC) by IHC staining with the FDA approved clone 28-8 (Dako) are extremely scant. Tumor infiltrating lymphocytes (TILs) have been associated with response to PD-1/PD-L1 inhibitors. In this study, we examine the expression of PD-L1 using clone 28-8 and TILs in invasive BC.

Design: PD-L1 staining was performed on tissue microarrays constructed from primary invasive breast cancer resection specimens of 481 patients diagnosed between 2004 and 2016 and treated in our institution. All patients received standard therapy without PD-1/PD-L1 inhibitors. Stromal TILs (sTILs) was evaluated on corresponding whole slide sections according to the International Working Group

guidelines. For PD-L1, membranous staining of ≥1% was considered positive. For sTILs, ≥5%, ≥10% and ≥20% were used as cutoffs. Statistical analysis was performed to assess the association of PD-L1 and sTILs with clinicopathological features and clinical outcome in the entire cohort, pretreatment and post neoadjuvant chemotherapy (NACT) specimen cohorts using SPSS software.

Results: Positive PD-L1 staining was detected in 11.9% (57/481) of tumors and was highest in the triple-negative subtype (23/104; 22.1%). As shown in Table 1, positive PD-L1 was associated with higher histologic grade, higher sTILs, ER(-), PR(-) and triple-negative status. Regardless of cutoff selection, higher sTILs were associated with younger age, ductal subtype, higher histologic grade, lower pT stage, ER(-), PR(-), HER2(+) and triple-negative status (data with 10% cutoff are shown in Table 1). When positive PD-L1 and higher sTILs were combined, histologic grade, ER, PR and triple-negative status remained significant features. For most features, the significance was not affected when the cohort were divided into pretreatment and post NACT specimens (Table 1). Follow-up time ranged from 0.4 to 124.3 months (median: 23.0 months). Positive PD-L1 was associated with worse disease specific survival (DSS) in the pretreatment cohort (P=0.023), and not with overall survival, disease-free survival and distant disease-free survival in any cohort. sTILs or combined PD-L1 and sTILs was not associated with survival in any cohort.

Table 1. Association of PD-L1 (clone 28-8) and sTILs with clinicopathological features

Clinicopathologic features	P-value		
	PD-L1 (28-8) (pos vs neg)	sTILs (high vs low)	PD-L1 28-8/ sTILs (pos/high vs others)
Age [N=481 (< 50 yrs: 146; ≥ 50 yrs: 335)]	0.542	0.020*	0.582
Neoadjuvant chemotherapy [N=481 (Yes: 144; No: 337)]	0.644	0.438	0.462
Histology subtype [N=480 (IDC: 406; ILC: 53; Mixed IDC/ILC: 16; Metaplastic carcinoma: 5)]	0.319	<0.001*	0.241
Histologic Grade [N=479 (G1: 73; G2: 221; G3: 185)]	<0.001*#	<0.001*#	<0.001*#
sTIL [N=475 (<5%: 239; 5-9%: 110; 10-19%: 47; ≥20%: 79)]	<0.001*#	N/A	N/A
ER [N=481 (Neg: 111: Pos: 370)]	<0.001*#	<0.001*#	<0.001*#
PR [N=480 (Neg: 169; Pos: 311)]	<0.001*#	<0.001*#	<0.001*#
HER2 [N=481 (Neg: 438; Pos: 43)]	0.623	0.040*	0.766
Receptor subtypes [N=481 (ER+/HER2-: 334; HER+: 43; TN: 104)]	0.001#	<0.001*#	<0.001*#
Triple Negative [N=481 (Yes: 104; No: 377)]	0.001#	<0.001*#	<0.001*#
pT stage [N=481 (pT1: 250; pT2: 158; pT3: 57; pT4: 16)]	0.421	0.016#	0.530
pN stage [N=480 (pN0: 276; pN1: 132; pN2: 37; pN3: 35)]	0.278	0.360	0.331
pM stage [N=481 (pM0: 467; pM1: 14)]	1.000	0.373	0.613

P-values of <0.05 were considered statistically significant.

The P-values shown are for the entire cohort. The significant ones are in bold. *P-value was significant in the pretreatment specimen cohort; #P-value was significant in the post-neoadjuvant chemotherapy specimen cohort.

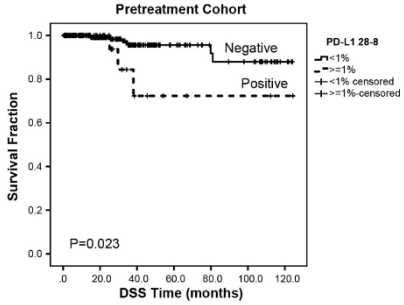


Figure 1. Kaplan-Meier curve of PD-L1 (28-8) and DSS.

Conclusions: PD-L1 (clone 28-8) was expressed in 11.9% of invasive BCs not treated with PD-1/PD-L1 inhibitors. Its expression was associated with sTILs, histologic grade and receptor status. PD-L1 was associated with DSS in the pretreatment specimen cohort.

190 Nucleus Detection and Segmentation for Pathology Images Using Deep Convolutional Neural Network and Variational Autoencoder

Su Hai[1], *Manish Sapkota*[2], *Fujun Liu*[1], *Jinzheng Cai*[2], *Pingjun Chen*[1], *Lin Yang*[1]. [1]University of Florida, [2]University of Florida, Gainesville, FL

Background: An increasing incidence of cancer has been witnessed in recent years. Accurate patient stratification and treatment planning heavily relies on high-throughput and reproducible pathological screening. However, manual analysis is limited in both analysis throughput and reproducibility. Computer aided diagnosis provides promising alternatives to help pathologists make efficient and better decisions. Fast and accurate nucleus detection and segmentation is an essential prerequisite for many subsequent analyses (*e.g.,* nucleus counting, classification and proliferation estimation). The major challenges in nucleus detection and segmentation are efficiency and robustness against to significant variations in nucleus appearance. In this study, we present a very efficient nucleus detection and segmentation framework using fully convolutional neural network (FCN) with deep supervision and variational autoencoder (VAE).

Design: In this abstract, we present a novel nucleus detection and segmentation system based on FCN and VAE. The FCN is a VGG16 deep convolutional neural network with deep supervision. The FCN, trained using structural labels with 1 at the center of each nucleus and 0s at other pixels, is used for detection. The VAE, trained on the original data with their structured labels, is used for segmentation. To handle large variation in nucleus size, we trained a standard VGG16 network to predict the nucleus size. In testing, each nucleus is cropped into an image patch of the predicted size, the segmentation result is obtained by applying the VAE to the image patch.

Results: The proposed system is evaluated on the breast cancer pathology images from TCGA dataset. The FCN achieves F1 score of 96% for detection and the VAE achieves F1 score of 88% for segmentation. It is clear that the proposed method learns to capture the structure of the cell boundaries. Therefore, the true boundaries can be recovered in the presence of inhomogeneous intensity, and a better detection and segmentation performance is achieved. The proposed algorithm is very efficient and can segment out about 9000 nuclei in a 5000x5000 image in 22 seconds.

Conclusions: We have developed a novel nucleus detection and segmentation system based on fully convolutional neural network and variational autoencoder. The proposed method is very efficient and accurate. In addition, the framework is completely data driven and thus can be adapted to other pathological images.

191 Frozen Section of Sentinel Lymph Nodes in 702 Breast Cancer Patients Treated with Neoadjuvant Chemotherapy

Sabina Hajiyeva[1], *Tracy-Ann Moo*[1], *Maria A Friedlander*[1], *Maura A McCormack*[2], *Katherine Lopez*[1], *Michele Stempel*[1], *Monica Raiss*[3], *Andrea Barrio*[1], *Monica Morrow*[1], *Marcia Edelweiss*[4] [1]Memorial Sloan Kettering Cancer Center, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]Memorial Sloan Kettering Cancer Center, New York, NY, [4]MSKCC, New York, NY

Background: Assessment of Sentinel Lymph Nodes (SLN) by frozen section (FS) helps determine the need for axillary lymph node dissection at first surgery. After neoadjuvant chemotherapy (NAC), the intraoperative examination of SLN can be challenging. Our aim is to evaluate the false negative (FN) rate of SLN FS in breast cancer patients treated with NAC and determine if any histomorphologic factors are consistently associated with the FN results.

Design: Patients who had intraoperative SLN assessment after NAC were identified from a prospectively maintained institutional database from July 2008-July 2017. The results of SLN biopsy at intraoperative FS, on permanent paraffin embedded H&E stained sections, and other associated clinical-pathological characteristics were recorded.

Results: We identified 711 SLN FS cases after NAC from 702 patients (9 of which had bilateral surgery). There were 522 negative, 181 positive, and 8 deferred FS results for intraoperative SLN biopsy. Twenty-eight out of 522 negative cases had positive results on permanent sections, (false negative rate=5.36%). Number of lymph nodes sampled for patients with FN result ranged from 1 to 10; median 4 lymph nodes. There were no false positive FS results. The deferred rate was 1.1%. Out of the 8 deferred cases 5 were confirmed positive on permanent section and 3 were negative. Eighty-nine (89%) of FN cases had metastatic foci identified exclusively on permanent H&E sections. See table with summary of the FN results.

Method of detection	Frozen Section-False Negative (n=28) (FN rate 5.6%)									
	ITC (n=9)	Micrometastasis (n=12)	Macrometastasis (n=7)	Ductal (n=23)	Lobular (n=2)	Mixed features (n=3)	ER-HER2- (n=2)	ER-HER2+ (n=1)	ER+HER2- (n=22)	ER+HER2+ (n=3)
Permanent and/or IHC only	9	9	7	21	1	3	2	1	19	3
FS lookback +H&E	0	3	0	2	1	0	0	0	3	0
p-value	0.4			<0.0001			<0.0001			

Conclusions: The false negative rate of intraoperative frozen section examination after NAC is low (5.6%). Most FN cases were of ductal phenotype, ER+HER2- and had metastatic foci identified exclusively on permanent H&E sections and not on original FS re-review. This may be associated with under sampling of SLN at the time of FS. Intraoperative FS is a reliable modality to assess the presence of SLN metastases in NAC treated patients.

192 High Tumor Infiltrating Lymphocyte Involvement in Breast Cancer in African American Women

Allison Hall, Durham, NC

Background: There is evidence that breast cancers in African American women differ from those in Caucasian women in terms of grade and molecular subtype. To date, the question of whether race is also associated with differences in tumor infiltrating lymphocytes (TILs) in breast cancer has not been specifically addressed.

Design: The Duke Cancer Registry was used to identify consecutive cases of breast carcinoma in African American women from December 2016 to May 2017 (n=39). A similar number of consecutive cases from Caucasian women were identified over the same time period (n=36). The degree of TIL involvement in each case was determined using the method described by the International TILs Working Group. For subjects who had undergone neoadjuvant chemotherapy, pre-treatment needle core biopsies were examined. In all other cases, surgical specimens were used. Demographic information and tumor characteristics were extracted from the subjects' medical records.

Results: Tumors from African American subjects were less likely to express ER (72% vs 92%) and more likely to be triple negative (TN) (26% vs 6%) than tumors from Caucasian subjects. The average tumor grade was higher for African American subjects than for Caucasian subjects (2.4 vs. 2.0).

Overall, there was a significantly higher level of TIL involvement in the African American group, compared with the Caucasian group (16% vs 7%, p = 0.02, t-test). There was also a trend toward higher levels of TILs in the African American group when the tumors were divided by molecular subtype (see table). Among grade 2 tumors, there were significantly more TILs in tumors from African American subjects than in tumors from Caucasian subjects (10.4% vs 4%, p= 0.05, t-test).

Average TIL Involvement

	All cases	Luminal A	Her2+	TN	Grade 1	Grade 2	Grade 3
African American	15.9%	9.3%	16.7%	30.7%	10.2%	10.4%	21.8%
Caucasian	7%	6.8%	8.2%	7.5%	5.1%	4%	14.8%

Conclusions: This study demonstrates a higher level of TIL involvement in breast carcinomas in African American women than in Caucasian women. This may be partially explained by the higher prevalence of TN tumors in the African American population, as TN tumors are known to have higher rates of TIL involvement. However, even within tumor subgroups, there are consistently higher levels of TILs among African American subjects. These findings suggest that there may be differences in TIL response in patients of different races that are distinct from the differences based on established tumor subtypes. The presence of a high degree of TIL involvement in breast cancers in African American women also indicates that evaluation of immune-based therapies in this population may be warranted.

193 Tumor Size in Breast Carcinoma: How Important is the Gross Measurement?

Ameer Hamza[1], *Ramen Sakhi*[2], *Ahmed Alrajjal*[2], *Warda Ibrar*[2], *Jacob Edens*[2], *Sajad Salehi*[2], *Ian Anderson*[2], *Daniel Ockner*[3]. [1]St. John Hospital & Med Ctr., Detroit, MI, [2]St. John Hospital and Medical Center, [3]Bloomfield Hills, MI

Background: The staging of breast carcinoma is mainly dependent on tumor size and lymph node status. Small increments in tumor size can upstage the patient. The accurate determination of the size is therefore critically important. Although the final staging is done on microscopic size, pathologists have no choice but using the gross measurement in considerable number of cases.

Design: We investigated the concordance between gross and microscopic measurements of breast carcinoma and factors affecting this concordance. This study was a retrospective review of surgical pathology reports of breast carcinomas. Data was collected for 470 cases. We used ± 2mm size as a cut off for concordance.

Results: Gross and microscopic size was exactly the same 33.3% cases. Gross and microscopic size difference was within ± 2mm in 55.5% cases. Despite the size difference, stage classification ended up being same in 68.3% cases. Gross measurement over estimated stage in 16.7% cases and underestimated it in 15% cases. The concordance was significantly higher for those tumors in which final pathologic tumor size was greater than 2 cm (≥ pT2) as compared to those less than or equal to 2 cm (≤ pT1) (p = < 0.0001). Higher proportion of mastectomy (60.9%) specimens had concordance as compared to lumpectomy specimens (51.6%). Tumor site, tumor type, neoadjuvant therapy, specimen weight, formalin fixation time and grossing person (resident vs. pathology assistant) had no significant effect on the concordance (Table 1 and 2).

Table 1: Proportion of concordant cases among different study groups.

Study Group	Proportion of concordant cases	p-value
Tumor Site		
Upper outer quadrant	55.7%	0.75
Upper inner quadrant	54.0%	
Lower inner quadrant	58.3%	
Lower outer quadrant	55.2%	
Central	77.8%	
Tumor Type		
Invasive ductal carcinoma	54.5%	0.41
Invasive lobular carcinoma	64.8%	
Ductal carcinoma in situ	44.4%	
Other tumors	55.6%	
Neoadjuvant therapy		
Yes	47.5%	0.27
No	56.3%	
Grossing person		
Resident	56.4%	0.72
Pathology assistant	54.7%	

Table 2: Correlation coefficient with respect to specimen weight and formalin fixation time.

Specimen Weight	Lumpectomy Specimen	Mastectomy Specimen
Spearman's rho correlation coefficient	-0.145	-0.040
p-value	0.19	0.60
Formalin fixation time		
Spearman's rho correlation coefficient	0.043	0.040
p-value	0.57	0.64

Conclusions: Stage classification based on gross tumor size and microscopic size is different in nearly one-third (31.7%) cases. Gross tumor size is critically important in accurate staging.

194 Pathologic Complete Response Rate and Clinical Outcome of Metaplastic Breast Carcinoma After Neoadjuvant Chemotherapy

Min Han[1], *Huina Zhang*[1], *Beth Clark*[2], *David Dabbs*[1], *Rohit Bhargava*[1]. [1]Magee-Womens Hospital of UPMC, Pittsburgh, PA, [2]University of Pittsburgh, Pittsburgh, PA

Background: Metaplastic carcinoma of the breast is often associated with poor prognosis partially due to the lack of effective therapy. We sought to investigate the response of metaplastic carcinomas to neoadjuvant chemotherapy (NACT) and identify tumor characteristics associated with pathologic complete response (pCR) and associated clinical outcome.

Design: One-hundred eight cases of metaplastic breast carcinoma were identified in the past 10 years. Thirty (28%) received NACT which formed the basis of this study.

Results: Average age of the cohort was 53 years. Average pre-therapy tumor size was 4.6 cm. Six (20%) were weakly ER+ with average H-score of 5. All tumors were negative for PR. Two (7%) were HER2 positive. Average Ki-67 labeling index was 72%. Most (25/26 or

96%, data not available in 4) of the tumors were nuclear grade 3. The most predominant subtype was matrix producing (12 cases, 40%), followed by squamous (10 cases, 33%), and others including spindle and mixed (8 cases, 27%). Most patients (57%) received Adriamycin, Cyclophosphamide and Taxane (ACT) chemotherapy regimen. Of the 14 cases with pre-therapy lymph node (LN) core biopsy, 9 (64%) were positive. Ten of 29 cases (34%, data not available on 1) showed metastatic tumor in post-therapy lymph nodes.

Five (17%) patients achieved pCR. None of the variables predicted for pCR (all p-values >0.05). Average follow up period was 48 months. There were 7 recurrences, 1 in pCR group and 6 in "no pCR" group (p=1.0). There was one death in the pCR group and 8 in the "no pCR" group (p=1.0).

Patient with positive pre- and post-therapy LNs showed statistically significant higher recurrence rate, 100% versus 38% with pre (p=0.0310) and 71% versus 23% (p=0.0302) with post-therapy LNs. The mean age of patients who died during follow up was 62 years compared to 51 years for patients who were alive (p=0.0385). Survival analysis showed increased risk of death with positive pre and post-therapy LNs (relative risk of 3.8; 95% CI: 1.1 to 12.0, p=0.0235). Other variables were not significantly associated with recurrence or survival.

Table 1. Patient and tumor characteristics in relation to response to neo-adjuvant chemotherapy, recurrence status and patient survival.

	pCR			Recurrence			Survival	
	Yes (n=5)	No (n=25)	p	Yes (n=7)	No (n=23)	p	Alive (n=21)	Dead (n=9)
Age (mean)	57	54	0.62	60	52	0.17	51	62
Pre-therapy tumor size (cm)	2.6	4.9	0.13	6.3	4.0	0.10	4.0	5.9
ER+	0	6/25 (24%)	0.55	3/7 (43%)	3/23 (13%)	0.12	3/21 (14%)	3/9 (33%)
PR+	0	0	NA	0	0	NA	0	0
HER2+	1/5 (20%)	1/25 (4%)	0.31	0	2/23 (9%)	1.0	2/21 (10%)	0
Ki-67 (mean)	64%	73%	0.36	76%	70%	0.49	70%	74%
Grade 3	5/5 (100%)	20/21 (95%)	1.0	7/7 (100%)	18/19 (95%)	1.0	16/17 (94%)	9/9 (100%)
Matrix producing subtype	3/5 (60%)	9/25 (36%)	0.36	3/7 (43%)	9/23 (39%)	1.0	9/21 (43%)	3/9 (33%)
Squamous subtype	0	10/25 (40%)	0.14	3/7 (43%)	7/23 (30%)	0.66	7/21 (33%)	3/9 (33%)
ACT regimen	3/5 (60%)	14/25 (56%)	1.0	5/7 (71%)	12/23 (52%)	0.43	11/21 (52.4%)	6/9 (67%)
Pre-therapy LN+ (n=14)	1/2 (50%)	8/12 (67%)	1.0	6/6 (100%)	3/8 (38%)	0.03*	3/8 (38%)	6/6 (100%)

*Statistically significant; pCR: Pathologic complete response; ACT: Adriamycin, Cyclophosphamide and Taxane; LN: Lymph node; NA: Not available.

Conclusions: The pCR rate of metaplastic carcinoma is one-half the pCR rate of other "triple negative" breast cancers. No clinical, morphologic or immunohistochemical variable is predictive for pCR. pCR did not translate into improved survival in this cohort. However, similar to other breast cancers, positive lymph node status is an important determinant of recurrence and survival in metaplastic carcinoma.

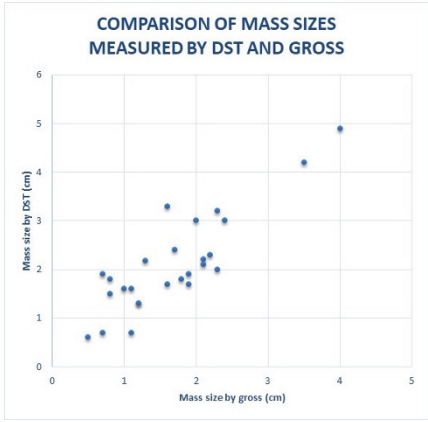
195 Application of Digital Specimen Tomography in Identifying Largest Dimension of Invasive Breast Carcinomas

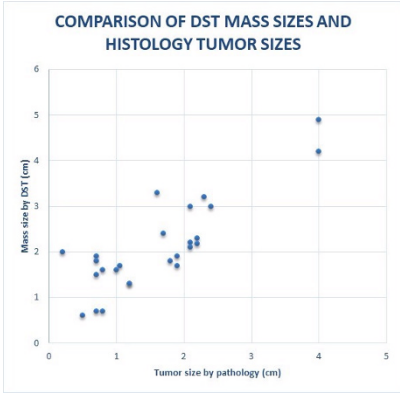
Lisa Han[1], *Xiao Han*[1], *Kirti Kulkarni*[2], *Xiaochuan Pan*[2], *Jeffrey Mueller*[3]. [1]University of Chicago, Chicago, IL, [2]University of Chicago, [3]Chicago, IL

Background: Pathological evaluation of breast specimens relies critically on gross and radiographic findings. Digital Specimen Tomography (DST) is a new tool that is sufficiently compact and portable for potential routine use in the gross room for breast specimens. It yields a full 3D image of tissue with sub 100-micron isotropic resolution in under 4 minutes. Mass lesions can be visualized and measured in all orthogonal views. Our preliminary study evaluates DST's utility in determining largest dimension of invasive carcinoma, compared to gross examination findings, and correlating with final reported pathologic tumor dimension.

Design: We performed DST on 25 fresh, oriented breast lumpectomies with single or multiple foci of invasive carcinomas before specimens were measured, inked and serially sectioned. Grossly identified lesions were sampled using conventional grossing techniques without consideration of DST findings. DST images were then reviewed, assessed for largest lesion(s) dimension, which were then compared separately with tumor size as identified by pathologic assessment, and with gross mass size. Mean square error (MSE) was computed to evaluate correlation of DST with gross and histology results.

Results: DST aided in identifying the shape and dimensions of mass lesions including tumor, as well as clip, previous biopsy site and/or calcifications in cases that were difficult to identify on gross inspection alone. DST-measured lesion size showed better correlation with grossly measured mass size than with histologic tumor size ($MSE_{DST-GROSS}$=0.657, $MSE_{DST-PATH}$=0.741) (Fig. 1 and 2). Lesion size by DST was within 2 mm of histologic tumor size in 50% and overestimated in 50% of cases. In this series, DST did not underestimate tumor size, suggest DST may have higher sensitivity than specificity in detecting dense breast lesions.





Conclusions: DST is an effective, innovative adjunct to conventional grossing of breast excisions with ability to identify mass lesions which warrants sampling during grossing. Because it recapitulates the 3D shape to potential tumors, it can aid in initial gross assessment of breast specimens. Ongoing studies are conducted to detail non-invasive components identified by DST.

196 Invasive Lobular Carcinoma with an Unusual Immunophenotypic Profile

Matthew G Hanna[1], *Anne Grabenstetter*[2], *Dara Ross*[3], *Lee Tan*[4]. [1]Memorial Sloan Kettering Cancer Center, New York, NY, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]New York, NY, [4]Memorial Sloan-Kettering, New York, NY

Background: Invasive lobular carcinoma (ILC) comprises about 10% of all invasive breast cancers. While the majority of ILC are of classic (CL) type, the other major variants are pleomorphic (PL) and solid (SOL) types. CL is generally reported as having favorable prognostic features (estrogen receptor (ER) positive and human epidermal growth factor receptor (HER2) negative). In contrast, the PL and SOL types are reported as more aggressive. This study investigated the immunophenotype and clinical features of CL, PL and SOL in a large Cancer Center.

Design: Patients (pts) with ILC were identified upon retrospective review of our institutional database from 2009 to 2017. Demographic, morphologic, and immunophenotypic data were obtained from pathology reports to determine the clinicopathologic profile of the 3 predominant variants of ILC.

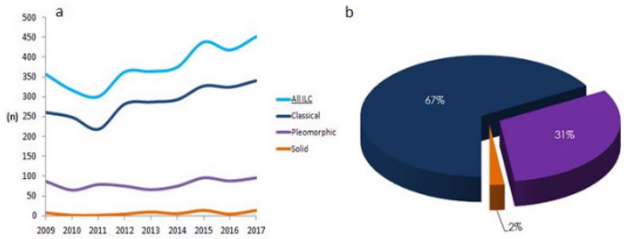
Results: A total of 1844 pts (1944 tumors) with ILC were identified, with a median follow up of 49.2 months. The majority of cases were classified as CL (67%, 1297/1944). Additional subtypes included: PL (31%, 608/1944) and SOL (2%, 39/1944). The median age of onset was: CL, 59 years; PL, 61 years; SOL, 67 years (Table 1). The median tumor size was: CL, 1.6 cm; PL, 2.4 cm; SOL 3.3 cm. The diagnosis of CL has increased over time at our institution, showing an average increase of 3.4% per year. There was no corresponding increase of PL or SOL (Figure 1). All ILC variants (CL, PL, SOL) showed high frequency of ER positivity with 99%, 85% and 98% respectively. PL cases had the highest frequency of HER2 positivity with 9% and SOL was HER2 positive in 3%. No CL cases were HER2 positive. 6% of PL cases were ER, PR and HER2 negative (triple negative) and this was not seen amongst the CL or SOL cases. Nodal metastases were seen in 30% of CL cases, 70% of PL and 69% of SOL. N3 (metastasis in 10 or more lymph nodes) was seen in 15% of PL and SOL and 5% of CL.

Figure 1: Number and Distribution of ILC
a, ILC from 2009-2017; b, Morphologic distribution of ILC

Table 1. Summary of Clinicopathologic Features

		Classical	Pleomorphic	Solid
	n	1297	608	39
	Age (median)	59	61	67
	Tumor size (cm, median)	1.6	2.4	3.3
	ER+/HER2-	99%	85%	98%
	ER+/HER2+	0%	7%	0%
	ER-/HER2+	0%	2%	3%
	ER-/HER2-	0%	6%	0%
Lymph Node Status	N0	70%	30%	31%
	N1	18%	30%	31%
	N2	7%	25%	23%
	N3	5%	15%	15%

		Classical	Pleomorphic	Solid
	n	1297	608	39
	Age (median)	59	61	67
	Tumor size (cm, median)	1.6	2.4	3.3
Hormone Receptor Status	ER+/HER2-	99%	85%	98%
	ER+/HER2+	0%	7%	0%
	ER-/HER2+	0%	2%	3%
	ER-/HER2-	0%	6%	0%
Lymph Node Status	N0	70%	30%	31%
	N1	18%	30%	31%
	N2	7%	25%	23%
	N3	5%	15%	15%



Conclusions: In this series, PL and SOL make up 33% of all reported ILC cases. The diagnosis of CL has increased over time which may be related to more screening and the improved sensitivity of imaging. Our data shows that PL can be triple negative in up to 6%, a finding not seen in SOL and CL. As expected, no CL was HER2 positive in this series. This is in contrast to the incidence of HER2 positivity in 9% and 3% of PL and SOL, respectively. These immunophenotypic features and higher stage at presentation reflect the more aggressive nature of PL and SOL variants.

197 Clinicopathological, immunohistochemical and molecular correlation of neural crest transcription factor Sox10 expression in triple negative breast carcinomas

Aparna Harbhajanka[1], *Satyapal Chahar*[1], *Hannah Gilmore*[2]. [1]University Hospitals Cleveland Medical Center, Cleveland, OH, [2]University Hospitals Case Medical Center, Case Western Reserve University, Cleveland, OH

Background: The transcription factor Sox10 mediates the differentiation of neural crest–derived cells and Sox10 immunohistochemistry (IHC) is used clinically primarily to support the diagnosis of melanoma. Sox10 expression has been previously documented in benign breast myoepithelial cells. However there is limited literature on its expression in triple negative breast carcinoma(TNBC). The aim is to study the clinical, pathologic and molecular profiles of SOX10+ tumors in TNBC.

Design: Tissue microarrays of TNBC were evaluated for SOX-10 and AR expression by IHC (clone SP107, Ventana Benchmark Ultra) in 48 cases. SOX10 and AR positivity was defined as greater than or equal to 10% staining in tumor nuclei. SOX10 expression was correlated with clinical and pathologic features such as age, grade, and stage. Gene expression was analyzed on RNA extracted from FFPE specimens with Affymetrix 2.0 HTA. Pietenpol TNBC molecular subtypes were calculated using the online TNBC type tool. Co-expression of SOX10 with other hormone-related proteins including AR, WT1, gross cystic disease fluid protein-15 (GCDFP-15) and GATA transcription factor 3 (GATA3) were evaluated.

Results: The mean age was 59.38 (range 28-90 years). Overall, 37.5% of cases 18/48 were SOX 10 positive. There was no association between SOX10 expression and age, grade or stage of patients. SOX10 positivity was most common in BL1 molecular subtype followed by unstable molecular TNBC categories of breast cancer. 6 of 10 tumors (60%) that were classified as BL1 by the TNBC subtype analysis tool showed Sox 10 positivity. Tumors that were Sox 10+ were also identified in the BL2 (1/5), IM(1/6), M (1/4), MSL (2/8) and unstable molecular TNBC categories(5/8) with lower frequency than what was seen in the BL1 group.

There was negative correlation between Sox 10 and AR positive subtypes (p value <0.002). Sox10 expression was positively correlated with WT1 expression (p value, 0.05). Sox10 did not show significant correlation with GCDFP15 and GATA3.

Clinicopathological parameters	SOX 10 Negative	Sox 10 positive	Total	P value
Age((yr, mean ± SD)	58.17±14.56	58.83±13.626	-	0.63
Size(cm, mean ± SD)	2.7±1.9	3.1±2.7	-	0.076
TNBC subtypes				
BL1	4	6	10	0.273
MSL	6	2	8	
LAR	4	1	5	
IM	5	1	6	
BL2	4	1	5	
M	3	1	4	
UNS	3	5	8	
Tstage				
T1	10	8	18	.347
T2	11	4	15	
T3	3	4	7	
Nstage				
N0	16	12	28	.484
N1	5	3	8	
N2	0	0	0	
N3	2	0	2	
Recurrance				
absent	23	15	38	.722
present	7	3	10	

Table 3: SOX10, AR, WT1, GATA3 and GCDFP15 expression in TNBC patients

Immunohistochemical markers	SOX 10 Negative	Sox 10 positive	Total	P value
Androgen receptor				
<10%	15	17	32	.002
>10%	15	1	16	
WT1				
Negative	25	11	36	0.54
Positive	4	7	11	
GATA3				
Negative	9	7	16	.545
Positive	21	11	32	
GCDFP-15				
Negative	20	15	35	0.172
Positive	9	2	11	

Conclusions: SOX10+ phenotype is most common in BL1 subtype of TNBC followed by unstable molecular TNBC categories gene expression profiling. TNBC must be considered in the differential diagnosis of melanoma for Sox10-positive metastatic malignant neoplasm. Sox10 expression in the basal-like, unclassified triple-negative, and metaplastic carcinomas types along with negative correlation with AR expresssion supports the concept that these neoplasms show myoepithelial differentiation.

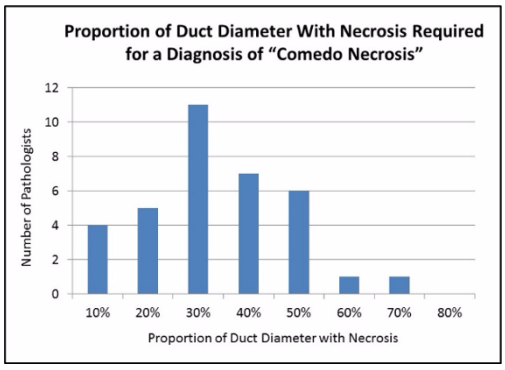
198 Variability in Diagnostic Threshold for Comedo Necrosis Among Pathologists: Implications for Patient Eligibility for Active Surveillance Trials of DCIS

Beth T Harrison[1], *Shelley Hwang*[2], *Ann Partridge*[3], *Alastair Thompson*[4], *Stuart Schnitt*[5]. [1]Brigham and Women's Hospital, Boston, MA, [2]Duke University School of Medicine, Durham, NC, [3]Dana Farber Cancer Institute, [4]MD Anderson Cancer Center, [5]Brigham and Women's Hospital; Dana Farber Cancer Institute, Boston, MA

Background: There are now three prospective, randomized clinical trials being conducted worldwide comparing active surveillance to standard management for women with "low risk" DCIS identified on core needle biopsy. In the U.S., the COMET trial is open to women ≥40 years old with ER+, low or intermediate grade DCIS diagnosed on core needle biopsy. The presence of comedo necrosis is a criterion for exclusion. However, the minimum amount of necrosis required for categorizing necrosis as "comedo" is not strictly defined in the COMET or other protocols. Potential variation among pathologists regarding the diagnostic threshold for comedo necrosis is a highly relevant issue in this setting but has not been assessed.

Design: Eight replicate histologic images of a single duct with low nuclear grade, solid pattern DCIS on which a central, solid, pink circle of various diameters was superimposed (representing 10%-80% of the duct diameter in 10% increments) were sent electronically to 35 experienced breast pathologists in the U.S. The participants were instructed to assume that the pink circles represented necrosis and were asked to report which of the 8 images represented the minimum amount of necrosis required for them to categorize the simulated necrosis as "comedo".

Results: Among the 35 pathologists, the threshold for comedo necrosis varied widely, from 10% to 70% of the duct diameter. The threshold proportion of the duct diameter with necrosis required for scoring "comedo necrosis" was 10% for 4 pathologists, 20% for 5, 30% for 11, 40% for 7, 50% for 6, 60% for 1, and 70% for 1 (see graph). There was no one threshold about which more than 30% of pathologists agreed met the minimal criteria for comedo necrosis. Variability in the threshold for comedo necrosis was seen even among pathologists working together at the same institution (ranging from 30% to 50%).



Conclusions: The threshold for diagnosing necrosis in DCIS as "comedo" is highly variable among experienced breast pathologists. While the most frequent threshold used for comedo necrosis was necrosis involving 30% of the duct diameter, the range of thresholds was broad. These findings highlight the need for a standard definition of comedo necrosis and awareness of this definition among pathologists. A low threshold for comedo necrosis could result in the exclusion from active surveillance trials of patients who may be suitable candidates, whereas a high threshold could result in inclusion of patients who may not be appropriate study subjects.

199 Non-Classical Lobular Carcinoma In Situ and Atypical Lobular Hyperplasia on Core Needle Biopsy: Correlation of Morphologic Features with Upgrade Rate.

Beth T Harrison[1], *Thing Rinda Soong*[1], *Faina Nakhlis*[2], *Tad Wieczorek*[3], *Kevin Hughes*[4], *Suzanne Coopey*[4], *Amy Ly*[5], *Catherine S Giess*[1], *Tari King*[2], *Stuart Schnitt*[6], *Susan Lester*[7]. [1]Brigham and Women's Hospital, Boston, MA, [2]Brigham and Women's Hospital, [3]Brigham and Women's Hospital, Boston, MA, [4]Massachusetts General Hospital, [5]Massachusetts General Hospital, Boston, MA, [6]Brigham and Women's Hospital; Dana Farber Cancer Institute, Boston, MA, [7]Brigham & Women's Hospital, Boston, MA

Background: Classical examples of lobular carcinoma in situ (LCIS) and atypical lobular hyperplasia (ALH), detected as an incidental finding on core needle biopsy (CNB), have been shown to rarely upgrade to invasive carcinoma on excision. In contrast, LCIS with non-classical features (including nuclear and cytoplasmic alterations, atypical biomarker expression and lymphocytic response) may present as a lesion on imaging and some cases are associated with invasive carcinoma on excision. The association of individual non-classical features with risk of upgrade to invasive carcinoma has not been well studied.

Design: All cases diagnosed as carcinoma in situ/atypical hyperplasia with "ductal and lobular features" or LCIS with "non-classical", "variant" or "pleomorphic" features on core needle biopsy since 2000 were identified by a key word search of pathology reports from 2 institutions. Cases were excluded if invasive carcinoma or DCIS were reported in the same biopsy or if an excision was not performed. Slides were scored for multiple features. Cases that displayed any non-classical features were included. Prevalence ratios (PR) by exposure categories were assessed via univariable and multivariable analysis using generalized linear models.

Results: Fifty-two cases of LCIS (50) or ALH (2) with non-classical features were included in the study. All but 3 cases exhibited multiple non-classical features. Twelve of the 52 cases (23%) were upgraded to invasive carcinoma on subsequent surgery. The prevalence of upgrade was significantly higher for lesions ≥0.5 cm in linear extent (35.7%; p=0.02) and those associated with a stromal lymphocytic response (66.7%; p=0.003). Trends toward upgrade were also observed for lesions with a high nuclear grade (31.0%), apocrine features (35.3%) and HER2-overexpression (42.9%) (see table). On multivariable analysis, the presence of a stromal lymphocytic response was the only feature associated with a significant risk of upgrade after controlling for confounders (adjusted PR: 4.0; 95% CI: 1.7-9.7).

CORRELATION OF HISTOPATHOLOGIC FEATURES OF NON-CLASSICAL LOBULAR NEOPLASIA ON BIOPSY WITH UPGRADE TO INVASIVE CARCINOMA				
		TOTAL CASES	UPGRADED CASES	
		N (%)	N (%)	p
NON-CLASSICAL LOBULAR NEOPLASIA		52 (100%)	12 (23.1%)	
NUCLEAR GRADE				
	HIGH	29 (55.8%)	9 (31.0%)	0.20
	HIGHER THAN USUAL/INTERMEDIATE	18 (34.6%)	1 (5.6%)	
	LOW	5 (9.6%)	2 (40.0%)	
LINEAR EXTENT >= 0.5 CM		28 (53.8%)	10 (35.7%)	0.02
FLORID GROWTH PATTERN		30 (57.7%)	7 (23.3%)	0.96
MODERATE TO ABUNDANT CYTOPLASM		36 (69.2%)	11 (30.6%)	0.08
APOCRINE FEATURES		17 (32.7%)	6 (35.3%)	0.15
SIGNET RING CELL FEATURES		14 (26.9%)	3 (21.4%)	0.86
MITOTIC ACTIVITY		37 (71.2%)	8 (21.6%)	0.83
CENTRAL NECROSIS		22 (42.3%)	6 (27.3%)	0.54
LYMPHOCYTIC RESPONSE		9 (17.3%)	6 (66.7%)	0.003
E-CADHERIN				
	ABERRANT	17 (32.7%)	4 (23.5%)	0.73
	NEGATIVE	27 (51.9%)	7 (25.9%)	
	NOT AVAILABLE	8 (15.3%)	1 (12.5%)	
ESTROGEN RECEPTOR				
	NEGATIVE	6 (11.5%)	1 (16.7%)	1.00
	POSITIVE (>1%)	33 (63.5%)	8 (24.2%)	
	NOT AVAILABLE	13 (25%)	3 (23.1%)	
HER2				
	POSITIVE (3+)	7 (13.5%)	3 (42.9%)	0.52
	EQUIVOCAL	12 (23.1%)	2 (25.0%)	
	NEGATIVE	15 (28.8%)	2 (13.3%)	
	NOT AVAILABLE	18 (34.6%)	4 (22.2%)	

Conclusions: In this large series, LCIS/ALH with non-classical features on CNB was associated with a substantial risk of upgrade to invasive cancer on excision (23%). Lesion characteristics associated with higher risk were identified, perhaps related to distinct biology. Further studies are necessary to better understand the biologic behavior of these morphologic variants of LCIS/ALH and their molecular underpinnings.

200 The Reproducibility and Clinical Utility of Tumor Infiltrating Lymphocytes Evaluation in Breast Core Biopsies in the Setting of Neoadjuvant Treatment

Bryce S Hatfield[1], *Michael Idowu*[2], *Henry R Bateman*[3], *Valentina Robila*[4]. [1]Virginia Commonwealth University Medical Center, Richmond, VA, [2]Virginia Commonwealth Univ Health System, Richmond, VA, [3]Virginia Commonwealth University, Richmond, VA, [4]Virginia Commonwealth University Health System, Richmond, VA

Background: The presence of tumor infiltrating lymphocytes (TILs) in breast cancer has been shown to correlate with pathological complete response (pCR) following neoadjuvant chemotherapy. While guidelines for quantitation of TILs on histologic sections are proposed, its implementation for clinical practice has not been achieved yet. Our study focuses on evaluation of stromal TILs on breast core biopsies pre-neoadjuvant chemotherapy, to provide further evidence that TILs may be used as an independent tumor biomarker. We assessed the pathologists interobserver reproducibility for scoring, TILs ability to predict outcome to treatment and a potential threshold for optimal response.

Design: Primary diagnostic breast needle core biopsies were independently evaluated by three pathologists, according to the recommendations of the International TILs group 2014. The stromal TILs quantitation score was subsequently correlated with tumor hormonal profile, pathologic response to therapy and long-term follow up. Only cases with neoadjuvant treatment, pathological and clinical follow-up information were included in the analysis. The Kendall's correlation coefficients were used for the inter-observer analysis and chi square was used for the statistical analysis.

Results: Of ninety-six patients meeting the above criteria at our institution between 2010-2012, fifty-five breast needle core biopsies were available for evaluation. The stromal TILs score was recorded on a continuous scale, with excellent interobserver reproducibility (K= 0.751). Overall, increasing stromal TILs correlated with a higher rate of pCR, and that was statistically significant for cases with more than 40% TILs (*p=0.001*; Table 1). However, correlation of TILs to response is variable depending on the tumor receptor status. Her2-positive carcinomas had the highest rate of pCR (56.3%), spanning the entire TILs score range (Table 2). For triple negative carcinomas, a score of >40% TILs correlated with pCR (*p=0.054*, Table 3). Of those, all biopsies with more than 60% TILs showed pCR. Lastly, 80% of tumors with luminal phenotype had low TILs (1-10%) and partial or no response to treatment.

All Evaluated Cases by Response and Score			
TILs %	pCR	Partial	nPR
>40	6 (75%)	1 (12.5%)	1 (12.5%)
11-40	4 (36%)	6 (55%)	1 (9%)
1-10	6 (16.6%)	23 (64%)	7 (19.4%)

Table 1. Correlation of the percent of stromal tumor infiltrating lymphocytes (TILs) with pathological response to neoadjuvant chemotherapy in all evaluated cases. (pCR- pathologic complete response; Partial- probable or definite response; nPR- no definite response).

HER2 Positive by Response and Score			
TILs %	pCR	Partial	nPR
>40	3 (100%)	0 (0%)	0 (0%)
11-40	2 (50%)	1 (25%)	1 (25%)
1-10	4 (44.4%)	4 (44.4%)	1 (11.1%)

Table 2: Correlation of the percent of stromal TILs with pathological response to neoadjuvant chemotherapy in Her2- positive breast carcinomas.

Triple Negative by Response and Score			
TILs %	pCR	Partial	nPR
>40	2 (66.7%)	1 (33.3%)	0 (0%)
11-40	0 (0%)	3 (100%)	0 (0%)
1-10	1 (16.7%)	5 (83.3%)	0 (0%)

Table 3: Correlation of the percent of stromal TILs with pathological response to neoadjuvant chemotherapy in triple negative breast carcinomas.

Conclusions: With adequate training, stromal TILs quantitation in breast core biopsies is highly reproducible between practicing pathologists. High levels of TILs predict improved response to neoadjuvant therapy, and a TILs score >40% is significantly linked to pCR in our cohort. Overall, the data further support TILs as a potential biomarker for clinical practice.

201 Can Tumor-Associated Macrophages in Ductal Carcinoma in Situ on Core Biopsy Predict Invasive Carcinoma on Excision?

Deepthi Hoskoppal[1], *Emily Reisenbichler*[2]. [1]Vanderbilt University, [2]Vanderbilt University, Nashville, TN

Background: Ductal carcinoma in situ (DCIS) diagnosed on core biopsy reportedly underestimates the presence of invasive breast cancer in subsequent excision in up to 49% of cases. Recent trials have explored surveillance of DCIS without complete excision but it is difficult to fully exclude an associated, un-sampled invasive focus. Histologic features such as mass lesions and comedo-necrosis are associated with higher risk of invasion, but tumor microenvironment, including tumor-associated macrophages, may play a role in the transition from in situ to invasive carcinoma. The presence of CD163 positive cells with DCIS has been associated with increased risk of progression to invasive carcinoma. The aim of our study was evaluate the role of DCIS-associated CD163 positive cells on core biopsy in predicting associated invasion on excision.

Design: Cases of DCIS diagnosed on core biopsy with follow up excision results were identified by pathology report search. Representative cases of low, intermediate and high nuclear grades, with or without subsequent invasion on excision were selected. Immunohistochemistry for CD163 was performed on core biopsies and positive intratumoral and stromal cells were quantified independently by 2 observers based upon the percentage of cells staining (score 0 <5%;1=5-25%;2=25-50%; 3=50-75%; 4=75-100%). Scores averaged between observers then were dichotomized into high and low expression based on mean score.

Results: 57 total biopsy cases of DCIS were identified of low (n=13), intermediate (n=21) and high (n=23) nuclear grade, 27 (47%) of which showed invasion on the subsequent excision specimen. Intratumoral CD163 scores ranged from 0-2 (mean 0.7). Stromal CD163 scores ranged from 0-3 (mean 1.3). Intratumoral and stromal CD163 levels were not significantly associated with the presence of subsequent invasion when evaluated as a whole group (p=0.36 and 0=0.47) nor when subdivided into low (p=0.36 and 0=0.17) intermediate (p=0.82 and 0=0.82) or high (p=0.09 and 0=0.68) nuclear grades There was no correlation between intratumoral CD163 content and DCIS grade (p=0.257). A trend for higher stromal CD163 expression was seen with higher grade DCIS though not statistically significant (p=0.178).

Conclusions: The presence of DCIS-associated CD163 positive cells at the time of diagnostic core biopsy does not predict the presence of a concurrent, un-sampled invasive tumor and is therefore not a helpful marker in selecting those patients who may safely forgo surgical excision.

202 Oncotype DX versus Magee Equation – the Number Game

Nihar H Hotchandani[1], *Toshi Ghosh*[2], *Jodi Carter*[3], *Beiyun Chen*[3]. [1]Mayo Clinic, [2]Mayo Clinic, Rochester, MN, [3]Mayo Clinic, Rochester, MN

Background: Oncotype DX breast cancer assay predicts distant recurrence in patients with ER+/HER2- breast cancer and costs >$4000 per assay. The Magee equations (developed by Magee-Womens hospital of UPMC) estimate the Oncotype DX recurrence score (RS) based on histologic tumor characteristics and biomarkers, and are free to use. Previous studies have found Magee equations to be a useful cost-saving tool for reliably predicting tumors with high (>30) or low (<11) RS. We evaluated the clinical relevance of the Magee equation as a screening tool for estimating RS in tumors referred for Oncotype Dx assay and investigated the pathologic features of the subset of tumors with discordant Oncotype DX RS and Magee estimated RS.

Design: All breast tumors referred for Oncotype DX assay in our institution during 2012-2014 were selected for the study. Tumor Nottingham grade (NG), Nottingham score (NS), size, ER, PR, HER2, and Ki67 (MIB1-clone, scored as % positive tumor nuclei by digital image analysis) were used to calculate the estimated RS using the modified Magee equation 1 (http://path.upmc.edu/onlineTools/mageeequations.html).

Results: Of 215 tumors in the study, 96% (206 tumors) had low to intermediate Oncotype DX RS (<31). 26% were NG 1, 66% were NG 2, and 8% were NG 3. The range of Magee estimated RS (9-29) was much narrower than that of Oncotype DX RS (0-56). Only

a small subset of tumors (N=22; 10.2%) have Magee estimated RS and Oncotype DX RS differing by more than 10 points. In this discordant subset, some tumors with lower Oncotype DX RS and higher Magee estimated RS had histologic features of intermediate-grade tumors and high Ki-67. Conversely, some tumors with higher Oncotype DX RS and lower Magee estimated RS (with low Ki-67) had prominent intra-tumoral reparative biopsy site changes (Table 1). The morphologic features of representative tissue blocks tested for Oncotype DX assay will be presented.

Case #	Oncotype RS	Magee RS	NG	Ki-67%	% tumor in block*	% biopsy site change**
1	0	15	1	18	80	0
2	0	13	2	8	80	0
3	8	21	2	30	70	10
4	10	22	2	49	70	10
5	22	10	1	6	40	10
6	28	17	2	7	30	40
7	36	17	2	20	60	20

NG - Nottingham grade. * % tumor in the block tested for OncotypeDX. ** % biopsy site changes in the same block.

Conclusions: At our institution, Magee equation-derived estimated RS and Oncotype DX RS show high concordance among the set of tumors clinically referred for Oncotype DX testing. As such, routine use of Magee equations is a useful cost-saving measure to decrease the need for Oncotype DX referral. Nevertheless, among cases sent for Oncotype DX testing, histopathologic tumoral correlation with Oncotype DX RS should be routinely performed and discordant cases may benefit from additional (re)-testing.

203 Evaluating the Expression of Mismatch Repair Proteins and Checkpoint Immune Markers and Their Association in Triple-negative and HER2-positive Breast Cancers

Yanjun Hou[1], *Hiro Nitta*[2], *Anil Parwani*[3], *Zaibo Li*[4]. [1]The Ohio State University, Columbus, OH, [2]Ventana Medical Systems, Inc., Tucson, AZ, [3]The Ohio State University, Columbus, OH, [4]Ohio State University Wexner Medical Center, Columbus, OH

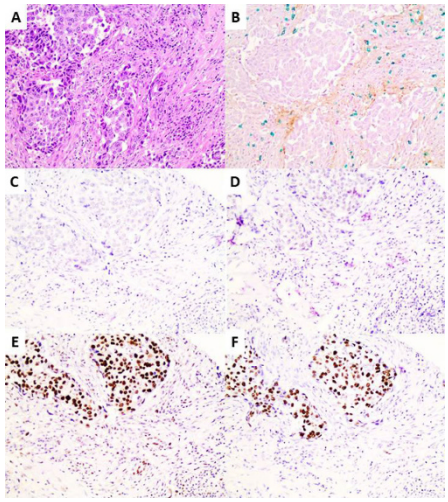
Background: Very few studies have investigated mismatch repair protein deficiency (dMMR) in breast cancer (BC). Unlike colorectal and endometrial cancer, the association between dMMR and PD1/PD-L1 has never been studied in BCs. Triple-negative BCs (TNBCs) and HER2-positive BCs are more aggressive than hormone receptor-positive/HER2-negative BCs and show higher levels of TILs. Given the recent FDA approval of Pembrolizumab for all solid tumors with dMMR, we aimed to examine the expressions of MMR proteins and checkpoint immune markers and their association in TNBCs and HER2-positive BCs.

Design: Immunohistochemistries (IHCs) with anti-MLH1, anti-PMS2, anti-MSH2 and anti-MSH6 were performed on pre-constructed tissue microarrays (TMAs) with 101 TNBCs and 197 HER2-positive BCs according to established protocols. Multiplex IHCs with anti-PD1, anti-PD-L1, anti-CD8 or anti-CD163 were also performed on TMAs according to established protocols.

Results: Thirteen cases (4.4%) showed dMMR, including 6 HER2-positive BCs (3%) and 7 TNBCs (6.9%). Eight cases had loss of MLH1 and PMS2, 2 had PMS2 loss, and 3 had MSH6 loss. Patients with dMMR were significantly younger than patients without dMMR. There was no difference regarding Nottingham grade, T staging and lymph node metastasis between these two groups. PD-L1 expression in either tumor cells or immune cells was identified in 37% of all cases (19% of HER2+ BCs and 71% of TNBCs). TNBCs showed significantly higher percentage of PD-L1 and CD8 expression than HER2-positive BCs. Both PD-L1 and CD8 expressions were significantly enriched in dMMR cases (Table 1).

Table 1. PD-L1 and CD8 expression in dMMR BCs and normal MMR BCs.

	Total		dMMR		Normal MMR		p value
	#/ aver-age	%/ range	#/ aver-age	%/ range	#/ aver-age	%/ range	
Total cases	298		13		285		
Age (years)	53.9	27-88	45.5	33-58	54.3	27-88	0.046
Nottingham grade 3	221	74.2%	11	84.6%	210	73.7%	NS
T stage (>1)	149	50.0%	7	53.8%	142	49.8%	NS
Lymph node metastasis	139	46.6%	6	46.2%	133	46.7%	NS
Any PD-L1+	109	36.6%	9	69.2%	100	35.1%	0.012
PD-L1+ in tumor cells	22	7.4%	1	7.7%	21	7.4%	NS
PD-L1+ in immune cells	105	35.2%	9	69.2%	96	33.7%	0.009
Peritumoral CD8+	144	48.3%	11	84.6%	133	46.7%	0.007
Intratumoral CD8+	92	30.9%	8	61.5%	84	29.5%	0.014



Conclusions: MMR deficiency exists in a relatively low percentage of BCs, when compared with endometrial or colorectal cancers. TNBCs show more dMMR and PD-L1/CD8 expression than HER2-positive BCs. MMR deficiency is associated with PD-L1 and CD8 expression in TNBCs and HER2-positive BCs.

204 Using Magee Equation to Predict Distal Recurrence Risk in Male Breast Carcinomas: A Single Institution Study

Yanjun Hou[1], *Gary Tozbikian*[2], *Anil Parwani*[3], *Zaibo Li*[4]. [1]The Ohio State University, Columbus, OH, [2]The Ohio State University, Columbus, OH, [3]The Ohio State University, Columbus, OH, [4]Ohio State University Wexner Medical Center, Columbus, OH

Background: Male breast cancer is rare, constituting less than 1% of all breast cancer cases. The etiology of male breast cancer is poorly understood. The most important risk factors are family history and mutations of *BRCA*. Invasive ductal carcinoma is the main type in men.

Magee equation(s) is derived by a linear regression analysis using several pathologic variables and semiquantitative immunohistochemical results of ER, PR, HER2, and Ki-67 to calculate a recurrence score. Previous studies demonstrated that Magee equation(s) effectively predict prognosis in female breast cancer. Here we presented a clinicopathologic study of male breast carcinoma with Magee equation recurrence score (RS).

Design: Thirty-two cases of male invasive breast cancers were studied. Clinical and pathological characteristics including patient's age, tumor grade, tumor size, lymph node status, ER, PR, HER2 amplification status and clinical outcome were collected. Magee equation RS was calculated for each case and correlated with clinical outcome.

Results: Mean age of the cohort was 63 years. Thirty-one cases were invasive ductal carcinoma and one was invasive lobular carcinoma. Three had Nottingham grade 1, 20 had grade 2 and 9 had grade 3. The average tumor size was 2.18 cm (range: 0.8 - 4.8). All cases were ER-positive and 27 were PR-positive (84%). Two cases (6%) were HER2-positive. Magee equation recurrent score (RS) was calculated for all cases: 12 with RS < 18, 19 with RS of 18-30, and 1 with RS > 30.

Thirty-one patients had follow-ups (mean: 76.7 months, range: 21.4

- 198.7 months). Among these 31 patients, all underwent hormonal therapy, but only 11 had chemotherapy (Adriamycin + Cytoxan). 27 had no recurrent disease, but four developed distal metastasis (2 in bone, and 2 in liver and lung). All four cases had high grade carcinoma (NG=3), and positive lymph nodes except 1 case with no lymph node status available. More importantly, all four cases had high Magee equation RS. The average Magee equation RS in these four cases was significantly higher than the average RS in other cases with no recurrent disease (29.6 vs 19.0, p = 0.00047).

Table 1. Clinicopathologic features and Magee equation RS of four male breast carcinoma cases with distal metastasis.

ID	Age	Type	NG	Size	ER	PR	HER2	Magee RS	HT	Che-mo	LN	Met	Time to met (months)	Death
1	58	Ductal	3	4.0	95%	1%	Neg	31.2	Yes	No	Pos	Bone	72.8	Yes
2	65	Ductal	3	4.7	90%	9%	Neg	29.4	Yes	Yes	Pos	Liver/ lung	21.4	Yes
3	79	Ductal	3	2.5	90%	20%	Neg	28.6	Yes	No	Pos	Liver/ lung	33.7	No
4	49	Ductal	3	1.0	80%	50%	Neg	29.3	Yes	Yes	NA	Bone	35.3	No

Conclusions: Male breast carcinoma shows similar distribution regarding histologic grades, biomarker status, and clinical staging. Magee equation may be useful to predict recurrence risk in male breast carcinoma patients.

205 Pathological Response Assessment to Neoadjuvant Chemotherapy in Breast Cancer Patients Utilizing Circulating Tumour Cells (CTCs)

Yanmei Huang[1], *Marie Fitzgerald*[2], *Cathy Spillane*[3], *Brendan Ffrench*[2], *Cathal O'Brien*[2], *Carmel Ruttle*[2], *Anna Bogdanska*[2], *Cara Martin*[3], *Dorinda Mullen*[4], *John Kennedy*[2], *Sharon O'Toole*[5], *John O'Leary*[6]. [1]Trinity College Dublin, Dublin, Ireland [2]Trinity College Dublin, [3]Trinity College Dublin, Dublin, [4]Dublin, Ireland, [5]Trinity College Dublin, Dublin, Ireland

Background: Detection and enumeration of Circulating Tumour Cells (CTCs) is associated with many cancers such as breast cancer, however, full prognostic and predictive power of CTCs for cancer cannot currently be harnessed, and the pathological complete response of neoadjuvant chemotherapy for breast cancer correlating with the CTCs is still not clear. The objective of this study was to assess the relationship between Circulating Tumour Cells (CTCs) and pathological response to neoadjuvant chemotherapy (NAC) in breast cancer patients.

Design: 24 patients were recruited and blood samples taken pre and post chemotherapy. CTCs were isolated using the ScreenCell device and stained using a modified Giemsa staining. CTCs were enumerated by 2 pathologists and classified as singles, doublets, clusters and microemboli. Counts were then correlated to the pathological response as measured by the Miller-Payne grading system. The associations between CTCs and clusters and pathological variables were evaluated with 2 or ANOVA tests performed in SPSS 24.0 statistic software.

Results: 83% of the patients had invasive ductal carcinoma (IDC) and 17% invasive lobular carcinoma (ILC). At baseline 83% of patients had CTCs present and only 4 patients were CTC negative. Median baseline count was 7 CTCs per 3mls whole blood. Post chemotherapy, 50% of the patients had an increase in CTC numbers. This change in CTC count/ in number of clusters did not correlate with the Miller Payne grade of response to chemotherapy. No significant association was identified between the number of CTCs in the pre-chemotherapy blood sample and clinical characteristics, including patient age, receptor status, tumour grade, disease type, or tumour size.

Conclusions: There was no correlation between the pre- and post-chemotherapy total number of CTCs/clusters and the Miller Payne grade. It is not enough to evaluate pathological response for neoadjuvant chemotherapy for Breast Cancer patients utilizing CTCs identified by Giemsa staining alone. Additional immunofluorescence is being carried out to further characterize the CTCs isolated pre and post chemotherapy. Long-term follow-up of these patients will determine the significance of CTCs in NAC breast cancer.

206 PD-L1 and P53 Expression Patterns in Invasive Ductal Carcinoma with Neuroendocrine Features

Xiao Huang[1], *Luis Blanco*[2], *K. P. Siziopikou*[1], *Jennifer Pincus*[3]. [1]Northwestern University, Chicago, IL, [2]Northwestern University Feinberg School of Medicine, Chicago, IL, [3]Northwestern Memorial Hospital, Chicago, IL

Background: Invasive ductal carcinoma with neuroendocrine features (IDCN) is an uncommon breast cancer subtype. PD-L1 is expressed in a number of tumors, including breast, and blockade of the PD1/PD-L1 axis has emerged as a promising new therapeutic option for cancer when PD-L1 is expressed in either the tumor cells or the associated lymphocytic infiltrate. Expression of p53 is commonly seen in triple negative breast cancers (TNBCs) and the patients with p53+ TNBCs have worse overall and disease-free survival than do patients with p53- tumors. In this study, we evaluated expression of PD-L1 and p53 as potential therapeutic and prognostic markers to characterize IDCN.

Design: The pathology database was searched for IDCN diagnosed between 2011 and 2017 (confirmed by chromogranin, synaptophysin, and/or CD56 positivity). Clinicopathologic data collected included age, tumor grade, tumor size, hormone receptor status, Ki-67 and p53 expression. PD-L1 expression in tumor cells (TCs) and tumor infiltrating lymphocytes (TILs) was assessed. TIL status was defined by the percentage of PD-L1 positive TILs in the tumor microenvironment: TIL0 (<1%), TIL1 (1-10%), and TIL2/3 (>10%).

Results: Between 2011 and 2017, 13 cases of IDCN were identified (mean age=62, range=38-82) at the time of diagnosis. Four tumors (31%) were grade 3, 7 were grade 2 (54%) and 2 were grade 1 (15%). Nine were luminal A (69%), two were luminal B (15%), and two were basal subtype (15%) by IHC. None of the tumor cells expressed PD-L1, but in 4 tumors (31%), (all grade 3, 2 of luminal B subtype and 2 of basal subtype), the associated TIL was positive (TIL2/3). The 9 remaining tumors were TIL0. Three cases (23%) showed P53 expression and all of these had coexpression of PD-L1.

Conclusions: IDCN is a heterogenenous group of tumors with a range of molecular subtype classifications. In our population, all the grade 3 IDCN demonstrated PD-L1 TIL2/3 and p53 positivity. Patients with high grade IDCN tumors may benefit from PD-L1 antibody treatment despite the poor prognosis associated with p53 expression. These findings suggest PD-L1 is a potential therapeutic target in this poorly classified subpopulation of breast cancers.

207 Correlation of MarginProbe with Final Pathological Diagnosis in Achieving Negative Margins in Breast Lumpectomy – A Single Institution Experience of 119 Cases.

Warda Ibrar[1], *Amanda Kupstas*[2], *Shelby Miller*[3], *Cheryl Wesen*[2], *Jeffrey S Falk*[4], *Daniel Ockner*[2]. [1]St. John Hospital and Medical Center, [2]St John Hospital and Medical Center, [3]St. John Hospital and Medical Center, Detroit, MI, [4]St John Hospital and Medical Center, Detroit, MI

Background: In breast conservation surgery, achieving negative margins is the ultimate goal. Recently, a handheld device MarginProbe, was approved for intra-operative margin assessment. At our institution, the utility of MarginProbe has shown a decrease in the re-excision rate from 18.3% to 9.1%. The purpose of this study is to stratify the results of MarginProbe in detecting benign vs atypical (including lesions with increased risk and LCIS) vs malignant lesions of the breast.

Design: We performed a retrospective chart review of 119 patients who underwent breast conservation surgery (lumpectomy) for early stage breast carcinoma (stage I and stage II). Patients included were over 18 years of age, had not received neo-adjuvant therapy, and had no prior surgery in the breast. The mean age of patients in this study was from 64.9 ±9.3 years. . The mean tumor size was 1.5 ± 1.04 (0.1 to 7cm). We found no statistically significant difference in tumor size for cases identified as positive or negative by Margin Probe. Six tissue sites for each case were evaluated by MarginProbe (medial, lateral, inferior, superior, anterior and deep), excluding the skin and pectoralis muscle, yielding total of 667 sites evaluated (n=714-47=667). 443 were negative by MarginProbe and were negative by final pathology. There were 224 additional shavings taken in which MarginProbe was considered to be positive and the final pathological diagnosis was analyzed.

Results: Positive MarginProbe results were divided into three categories based on increased risk of invasive carcinoma benign, atypical, and malignant. Of 224 positive MarginProbe shavings, data were missing for 31 cases and the rest 193 were evaluated. The rest were classified as follows: benign: 123(64%), atypical: 40 (21%) and, malignant: 30(15%). The frequency of detecting different types of lesions was calculated as follows:

Benign:

Fibrocystic changes	Fat necrosis	Benign calcifi-cations	Benign paren-chyma
41.8%	0.07%	8%	48.9%

Atypical/increased risk including LCIS:

ADH	ALH	PROLIF-ERATIVE BREAST DISEASE*	FLAT EP-ITHELIAL ATYPIA	LCIS
10%	12.8%	69.2%	0.05%	0.2%

*sclerosing adenosis, radial scar, and florid ductal hyperplasia.

Malignant:

DCIS	IDC	ILC	IDC+DCIS
63.3%	16.6%	16.6%	0.3%

Conclusions: MarginProbe is an effective device with very high sensitivity (95.6%) and negative predictive value (71.4%). Its utility can decrease the positive margins in breast conservation surgery. In malignant category 63.3% of true positive cases were DCIS and in increased risk category 69.2% cases were proliferative breast disease. The fibrocystic changes and benign calcifications constitute 50% of false positive readings. The utility of this device can save the trauma of re-excision and can be cost effective for both hospital and patients.

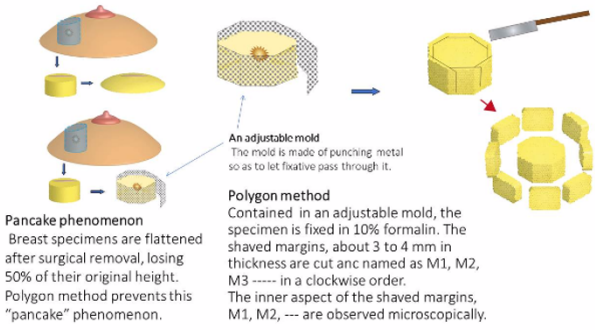
208 A New Method of Tangential Margin Assessment to Identify Ductal Carcinoma In Situ of the Breast that can be Controlled by Conserving Surgery Alone - A retrospective cohort study in Japan.

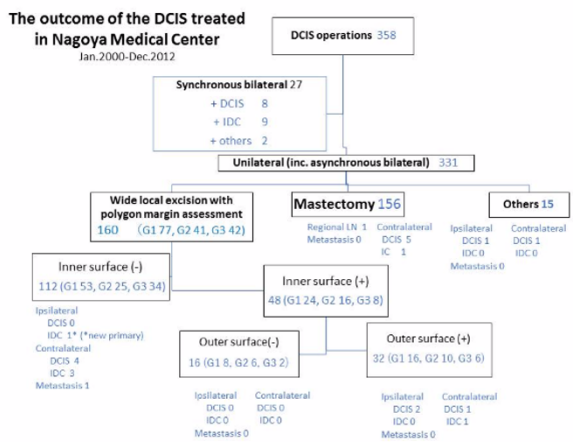
Shu Ichihara[1], *Suzuko Moritani*[2], *Tomonori Kawasaki*[3], *Mikinao Oiwa*[4], *Takako Morita*[5], *Takako Hayashi*[5], *Aya Kato*[5], *Noriko Ito*[1], *Akiko Kada*[5], *Tokiko Endo*[6], *Yasuyuki Sato*[5]. [1]Nagoya Medical Center, Nagoya, Aichi, [2]Shiga Medical University, [3]National Hospital Organization Nagoya Medical Center, Nagoya, Aichi, [4]Nagoya Medical Center, Nagoya, Japan, [5]Nagoya Medical Center, [6]East Nagoya Hospital

Background: Breast cancer screening frequently detects small, localized unicentric ductal carcinoma in situ (DCIS) of the breast, the potential candidates for breast-conserving surgery alone. However, the standardized and reproducible methods of margin evaluation for identifying the DCIS that can be controlled by excision alone are not yet established.

Design: In order to assess the relevant tangential margins in its entirety, we have developed a standardized system of margin evaluation employing a mechanism to maintain the polygonal-prism shape of the specimen during fixation in formalin bath.

Results: Our data base retrieved 160 women with primary unilateral DCIS who were treated by wide local excision during January 2000 and December 2012. By a new method of margin evaluation, 128 cases (80%) were margin negative and 32 cases (20%) positive. All conservatively treated DCIS except for one case were followed up without radiotherapy. With regard to ipsilateral local recurrence (ILR), one case (0.8%) of margin negative group developed invasive carcinoma and 2 cases (6.3%) of margin positive group developed DCIS in the preserved breast. Histopathologic, immunohistochemical and topographycal analysis indicates that one ILR case of margin negative group is a new primary whereas two ILR cases of margin positive group are true local recurrences.





Conclusions: Our retrospective cohort study indicates that there is no true local recurrence in the margin negative group evaluated by the new standardized system in spite of omission of radiotherapy. The margin negative group by the new system might not require radiotherapy after wide local excision.

209 The pathological response to vaccination in breast carcinoma is associated with the quantitative image analysis of the density of intratumoral T cell infiltrate in the pretreatment biopsies, and could be interfered by the immunosuppressive M2 macrophage blockade mechanism

Miguel Idoate[1], *Luis Mejías*[2], *Marta Abengozar*[2], *Maria Dolores Lozano Escario*[3], *Francisco Guillen-Grima*[4], *Marta Santisteban*[2]. [1]Clinica Universitaria de Navarra, Pamplona, Spain, [2]University of Navarra, [3]Madrid, [4]University of Navarra, Pamplona, Navarra

Background: Antitumor immunotherapy with dendritic cell vaccines (DCV) is a novel treatment of breast cancer. In the response to vaccines in breast cancer both effector and immunosupresive cells are involved. The relationship between vaccines and the intratumoral immune inflammatory infiltrate is not well understood. To assess the effect of immunotherapy on breast cancer, we have characterized and quantified the immune infiltrate in a wide range of paired biopsies of well-characterized clinical and molecular breast carcinomas treated by vaccine-neoadjuvant or only neoadjuvant therapies.

Design: We studied 160 paired pre and post-treatment biopsies of breast carcinomas classified as luminal A (24 cases), luminal B (30 cases) and triple negative (26 cases) subtypes, localized, stages II-III, corresponding to 80 patients, distributed in two groups: DCV-neoadjuvant, 36 cases or only neoadjuvant treatment (control group), 44 cases. Biopsies were characterized by immunohistochemistry against CD8, CD4, CD45 and CD163 (immunosuppressive M2 macrophages) and quantified by image analysis. Tumor regression was evaluated by Miller and Payne gradation score. A complete statistical study was carried out.

Results: The pathological response was slightly greater in the cohort of vaccinated than in control groups (p=0.03). This response was not associated with an increase of density of neither CD4 nor CD8 T cell infiltrates. Interestingly, as higher was the density of the either CD4 or CD8 infiltrates in the pretreatment biopsies higher was the tumor regression (p=0.003). On the other hand, the stromal immunosuppressive macrophage infiltration (CD163) formed dense aggregates in the tumor in all molecular subtypes, but especially in triple negative ones (p<0.0001). This inhibition mechanism was very strongly related with T stromal cell infiltration, because as higher was the density of the stromal T cells higher was the immunosuppressive infiltrates (p<0.001).

Conclusions: The dendritic cell vaccination is effective in the treatment of breast carcinoma because it is associated with pathological response, but it is not justified by an increase in the tumor stromal T cell infiltrate. This vaccination effect is related with the quantified density of the T cell infiltration in the pre-treatment biopsy. It is postulated that the immune effect of T cells could be interfered by the blockade of immunosuppressive mechanisms such as M2 macrophages

210 Histopathologic Features of Breast Tumors in Patients with Mutations in non-BRCA Moderate Penetrance Genes

Dorian Infantino[1], *Ira Bleiweiss*[2], *Kara Maxwell*[3], *Payal D Shah*[4], *Susan Domchek*[5], *Katherine Nathanson*[3], *Michael Feldman*[6], *Anupma Nayak*[7]. [1]Perelman School of Medicine & Hospital of the University of Pennsylvania, Philadelphia, PA, [2]Hospital of the University of Pennsylvania, Philadelphia, PA, [3]Hospital of the University of Pennsylvania, PA, [4]Hospital of the University of Pennsylvania, Philadelphia, PA, [5]Hospital of the University of Pennsylvania, [6]University of Pennsylvania, Philadelphia, PA, [7]Perelman School of Medicine & Hospital of the University of Pennsylvania, Philadelphia, PA

Background: With increasing availability of next generation sequencing and multigene platforms for testing hereditary predisposition to cancer, breast cancer patients are being identified who carry mutations in genes other than *BRCA1* and *2*. While the histologic characteristics of *BRCA1* mutation-associated breast carcinoma have been well described, including well circumscribed pushing borders, dense lymphocytic infiltrate, high histologic grade, and medullary features, the histology of breast carcinomas associated with other lower frequency high risk mutations have not been elucidated. In this study, we evaluated the histopathologic features of breast tumors associated with moderate penetrance non-BRCA genes.

Design: Retrospective study with review of H&E slides from breast cancer patients identified carrying known or proposed moderate penetrance breast cancer genes (CHEK2, ATM, PALB2, BLM, RAD51D & BARD1). A total of 31 patients with a diagnosis of invasive breast

carcinoma (n=26) or ductal carcinoma in-situ (DCIS) (n=5) were identified. Slides were available for review for 23 patients.

Results: The age of presentation was <50 years in 13 of 23 patients, with 7 being below 40 years. The distribution of hereditary mutations in 23 patients was as follows: CHEK2, 10; ATM, 6; PALB2, 2; BLM, 2; BARD1, 1; RAD51D, 2). A total of 25 tumors from 23 patients (2 patients with bilateral carcinoma) were reviewed. Twenty-three tumors were invasive whereas 2 were pure ductal carcinoma in-situ (DCIS). The histopathologic features of tumors including the ER/PR/HER2 receptor status for individual genes are summarized in Table 1.

Table 1- Clinicopathologic feature

	CHEK2	ATM	PALB2	BLM	RAD51D	BARD1	Total
N=25	10	7	2	3	2	1	25
Age, median	49	56	53.5	42.5	34	55	49
Histotype							
IDC, NOS	5	3	1	0	1	1	11
ILC	2	2	0	0	1	0	5
Mixed	1	0	0	1	0	0	2
Other	2	2	0	1	0	0	5
Pure DCIS	0	0	1	1	0	0	2
Tumor Differentiation							
PD	0	6	1	0	1	1	9
MD	7	1	0	1	0	0	9
WD	3	0	0	1	1	0	5
Associated lesions							
DCIS	8	7	1	1	1	1	19
LCIS	5	2	0	1	0	0	8
Radial Scar	3	0	0	0	0	0	3
Papilloma	3	1	1	1	0	0	6
TILs >50%	1	1	1	0	1	0	4
LVI	0	3	0	1	0	1	5
Multifocality	0	2	0	1	1	1	5
ER+PR±HER2-	6	6	1	1	1	0	15
HER2+	1	1	0	0	0	0	2
Triple negative	1	0	0	0	1	1	3

Conclusions: CHEK2 mutation was the most common aberration (43%), followed by ATM (26%). Overall, IDC, NOS was the predominant histological subtype. DCIS was a frequent precursor lesion (19 patients). Of note, none of the tumors associated with CHEK2 mutation were poorly-differentiated as opposed to ATM where 6 of 7 tumors were poorly differentiated. Radial scars and papilloma were commonly associated with CHEK2 tumors. Unlike BRCA1/2 associated tumors, the luminal (ER+PR±HER2-) subtype was predominant, and tumor infiltrating lymphocytes (TILs) were not a frequent feature.

211 Circulating Tumor Cells/cfDNA are an Independent Predictor of Overall Survival in Metastatic Breast Carcinomas: Analysis of 3,895 Patients

J. Bryan Iorgulescu[1], *Alarice Lowe*[1], *Jane Brock*[2]. [1]Brigham and Women's Hospital, Boston, MA, [2]Brigham & Women's Hospital, Boston, MA

Background: There is increasing excitement for the diagnostic and prognostic potential of circulating tumor cells/cell-free DNA (CTCs) in breast cancer patients, with preliminary series suggesting that CTC testing can be used to screen for recurrence and metastasis, serve as therapeutic biomarkers, and predict overall survival (OS); however, prior studies have been limited by small sample sizes. Herein we examine the prognostic utility of CTCs in a national cohort.

Design: The National Cancer Database comprises ~70% of newly-diagnosed cancers in the US and was queried for all breast invasive carcinomas from 2010-2014 who underwent CTC testing. Testing platforms included RT-PCR, immunomagnetic separation (IMS), and other. [2] and t-test were used as appropriate. OS was estimated by Kaplan-Meier methods, compared by log-rank; and risk-adjusted with Cox proportional hazards.

Results: 3,895 breast cancer patients underwent CTC testing: 50% by RT-PCR, 35% by IMS, and 15% by other methods. 28% of patients were positive for CTCs.

CTC-positivity was significantly associated (all p<0.001) with lobular histology, higher pT, nodal metastases (N1: 38%, N2: 35%, N3: 37%, vs N0: 22%), and distant metastases (M1: 37% vs M0: 21%); particularly, when compared vs CTC negative patients, with metastases to bone (75% vs 51%) and liver (32% vs 21%, p=0.04), but not brain (7% vs 10%, p=0.38) or lung (25% vs 31%, p=0.26) sites. There was no difference in age at diagnosis (mean 59 yrs, p=0.08) or histologic grade (p=0.65) and CTC-positivity. The proportion of patients presenting with metastatic disease did not differ between CTC-tested and non-tested cohorts.

CTC-positivity was an independent predictor of worse OS in survival analyses adjusted for age, treatment, pT, nodal status, histology, and receptor status in M1 cases (HR 6.2, 95%CI: 1.9-20.0, p=0.002) with a 5yr OS of 24% (vs 40% in CTC-negative cases, p=0.02); but not M0 cases (HR 1.0, 95%CI: 0.7-1.4, p=0.90) with a 5yr OS of 85% (vs 88%, p=0.09).

Conclusions: CTC detection in newly-diagnosed breast cancer patients was associated with a higher likelihood of nodal and distant metastatic (M1) disease. In patients with M1 disease, but not M0, CTC detection was independently associated with significantly worse OS in risk-adjusted analyses. Our results highlight the association with CTC-positivity and the presence of metastatic disease in a national cohort and the prognostic promise of CTC testing in metastatic breast cancer patients.

212 The 21-Gene Recurrence Score Adds Significant Prognostic and Predictive Value for Grade 3 Breast Carcinomas

J. Bryan Iorgulescu[1], *Susan Lester*[2], *Jane Brock*[2]. [1]Brigham and Women's Hospital, Boston, MA, [2]Brigham & Women's Hospital, Boston, MA

Background: The Onco*type* DX Recurrence Score (RS; GenomicHealth) is used by oncologists to identify patients who benefit from the addition of chemotherapy to endocrine therapy. However, it is possible that oncologists recommend chemotherapy for the majority of women with grade 3 breast carcinomas >1 cm (T1c or T2) without regard to RS due to the concerns about grade and size. This study was undertaken to determine national practices for ordering RS, treatment choices, and outcomes for this group of patients.

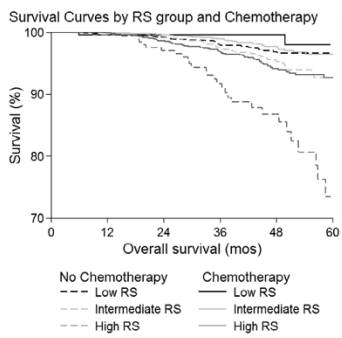
Design: The National Cancer Database includes about 70% of newly-diagnosed cancers in the US and was queried for T1c and T2, N0, M0, grade 3 ER+/HER- negative breast carcinomas from 2010-2014. Overall survival (OS) was evaluated by Kaplan-Meier, log-rank, and risk-adjusted proportional hazards.

Results: Of 21,028 grade 3 carcinoma patients with complete RS and chemotherapy data, 52% did not have RS determined. In this group, only 52% received chemotherapy in addition to endocrine therapy and demonstrated improved 5yr OS (94% vs 82%; p=<0.001).

RS testing for the other 48% of patients was associated with private insurance, academic hospitals, geography, lower comorbidity index, recent diagnosis, pT1c, radiotherapy, and breast conservation (all p<0.001). There were 27% low (<18), 41% intermediate (18-31), and 32% high (>31) scores.

For patients with low RS, 9% received chemotherapy, which was not associated with increased 5yr OS (98% vs 97%; p=0.40). In contrast, 55% of patients with intermediate RS and 89% of patients with high RS received chemotherapy and both groups had improved 5yr OS (97% vs 93%, p=0.003 and 93% vs 73%, p<0.001; respectively). These associations remained in analyses adjusted for patient, tumor, and treatment features.

Poorly-Differentiated T1c/T2 Cancers						
RS	n	No Chemotherapy		Chemotherapy		p value
		% patients	5-year OS (95% CI)	% patients	5-year OS (95% CI)	
Low	2,769	90.6%	96.6 (95.2-97.7)	9.4%	98.0 (90.4-99.8)	0.40
Intermediate	4,178	44.7%	92.7 (89.5-95.0)	55.3%	96.5 (94.7-97.7)	0.003
High	3,221	11.0%	73.4 (61.2-82.8)	89.0%	92.7 (90.6-94.3)	<0.001
Not performed	10,858	48.3%	81.7 (79.6-83.7)	51.7%	93.9 (92.6-94.9)	<0.001



Conclusions: Contrary to expectation, only 52% of patients with grade 3 T1c-T2 breast carcinomas without RS testing received chemotherapy. In contrast, 70% of patients with intermediate to high RS received chemotherapy, which was associated with improved OS. >90% of patients with low RS were spared chemotherapy. No benefit for chemotherapy was identified in low RS patients, although analysis was limited by few chemotherapy-treated low RS patients (n=261) and short follow-up (median 3 yrs). The data support that RS provides useful information for patients with grade 3 carcinomas by identifying patients who benefit from chemotherapy as well as patients unlikely to benefit. Treatment without reference to RS may lead to both over and undertreatment.

213 Prognostic Impact of Tumor-Associated Plasma Cells in Triple Negative Breast Cancer

Jabed Iqbal[1], *Joe Yeong*[2], *Aye Aye Thike*[3], *Bernett Lee*[4], *Jeffrey Chun Tatt Lim*[1], *Clara Ong*[3], *Noel Chia*[5], *Puay Hoon Tan*[6]. [1]Singapore General Hospital, Singapore, [2]Singapore General Hospital, Singapore, [3]Singapore General Hospital, [4]Singapore Immunology Network (SIgN), Agency of Science, Technology and Research (A*STAR), Singapore, [5]University of New South Wales, [6]Singapore General Hospital, Singapore

Background: Immune cell infiltrates play an important role in tumor response and majority of studies have focused on the role of tumor-infiltrating T-cell subtypes. The role of humoral immune response mediated by plasma cells in breast cancer has been understudied; immunoglobulin kappa C (IGKC), expressed in plasma cells, has been identified as one of the top genes of a prognostic B cell-metagene in breast cancer. This study aims to investigate the impact of plasma cell infiltration and IGKC mRNA in triple negative breast cancer (TNBC), an aggressive subtype devoid of estrogen and progesterone receptor expression as well as Her2 amplification.

Design: Two-hundred and sixty-nine (269) patients with TNBC diagnosed between 2003 to 2013 in Singapore General Hospital were retrospectively recruited in this study. Immunohistochemical expression of CD38 was analyzed in tissue microarrays; mRNA levels of immune response-related genes were studied with a quantitative, digital gene expression NanoString assay. Kaplan-Meier analysis and Cox regression analysis were used to determine the impact of CD38-positive plasma cells and mRNA expression on overall survival (OS), disease-free survival (DFS) and clinicopathological parameters. A p-value <0.5 was considered significant

Results: Multivariate analysis revealed that patients bearing TNBCs with high densities of $CD38^+$ plasma cells had better disease-free survival (HR=0.44; 95% CI 0.26-0.77; p=0.004). IGKC mRNA expression correlated specifically with the abundance of $CD38^+$ plasma cells (R=0.647); TNBC tumors with higher IGKC gene expression exhibited improved prognosis (overall survival, $p = 1.662E^{-04}$ and disease-free survival, $p=6.355E^{-05}$). Addition of intratumoral $CD38^+$ plasma cell density to clinicopathological features increased the prognostic value for both DFS ($\Delta LR^2 = 17.28$, $p= 1.71E^{-08}$) and OS ($\Delta LR^2 = 10.03$, $p= 6.32E^{-08}$), compared to clinicopathological features alone.

Conclusions: Our results demonstrate that plasma cell infiltration in TNBC impacts tumour progression and prognosis, thereby identifying a distinct subset of plasma cell-rich TNBC patients with favorable disease course. Moreover, the prognostic value of plasma cell density is independent of clinicopathological parameters, and of the densities of tumor-infiltrating lymphocytes. The important role of the humoral immune system warrants further studies and appropriate testing may be potentially incorporated in routine diagnostic work and cancer immunotherapy.

214 Molecular Profiling of Androgen Receptor Positive Triple Negative Breast Cancer

Jabed Iqbal[1], *Joe Yeong*[2], *Bernett Lee*[3], *Jeffrey Chun Tatt Lim*[1], *Aye Aye Thike*[4], *Puay Hoon Tan*[5]. [1]Singapore General Hospital, Singapore, [2]Singapore General Hospital, Singapore, [3]Singapore Immunology Network (SIgN), Agency of Science, Technology and Research (A*STAR), Singapore, [4]Singapore General Hospital, [5]Singapore General Hospital, Singapore

Background: Approximately ~20-30% of triple negative breast cancers (TNBCs) show androgen receptor (AR) expression, a subtype with relatively better survival among TNBCs. AR-positive TNBCs are less immunogenic, possibly due to the intrinsic immunosuppressive effect of AR or scant immune infiltrate. Expression of immunosuppressive immune checkpoint molecules or immune infiltrate composition is less studied in AR-positive TNBCs.

Design: Two hundred and forty-seven TNBC samples diagnosed between 2003 and 2013 in Singapore were subjected to a quantitative, digital gene expression NanoString assay to examine mRNA expression of a panel of 409 immune-stromal genes. Patients were divided into "AR-positive" and "AR-negative" groups based on AR protein expression. Sub-groups within AR-positive was determined using hierarchical clustering. Student t tests with Welch's correction was used to determine differentially expressing genes (DEGs) between the sub-groups. Multiple testing correction was done using the method of Benjamini and Hochberg. Ingenuity Pathway Analysis software (IPA) (Ingenuity System Inc, USA) was used for interpretation of data in the context of biological processes, pathways and networks. Disease free survival rate (DFS) and overall survival rate (OS) were correlated with the protein and mRNA expression. A p value <0.05 defined statistical significance in this study.

Results: A supervised classification was applied based on AR-positive and negative according to AR protein expression status. Profiling of 87 AR-positive TNBC samples revealed a sub-group of 37 cases showing significantly better disease-free survival (p= 0.046). and overall survival (p=0.023). Six genes were significantly differentially expressed *CTNNB1, UGT2B28, ALOX15B, LGALS9, HIST1H2AJ* and *CCNE2* were identified as DEGs (p adjusted<0.05), among which *LGALS9, HIST1H2AJ* and *CCNE2* were upregulated in the sub-group with better survival; IPA revealed TLR3 as significant upstream regulator (p<0.027) in the same sub-group of AR-positive TNBC.

Conclusions: *LGALS9* (Gal9) binding to the immune receptor T cell Ig and mucin-containing domain-3 (Tim-3) enhances the production of IFN- by NK cells. Conversely, higher doses of Gal-9 impair the cytotoxic function of NK cells in a Tim-3 independent manner. These data indicate a potential role of the TIM3 immune checkpoint molecule in TNBC particularly in AR-positive TNBC. Role of TLR3 also warrants further study in AR-expressing TNBC.

215 Mammary Myofibroblastoma: A Clinicopathologic Study of 27 Cases

Seema Jabbar[1], *Kavita Varma*[2], *Beth Clark*[3], *Yisheng Fang*[4], *Rohit Bhargava*[5], *Sunati Sahoo*[1]. [1]UT Southwestern Medical Center, Dallas, TX, [2]Magee Women Hospital, Pittsburgh, PA, [3]University of Pittsburgh, Pittsburgh, PA, [4]UT Southwestern Medical Center, [5]Magee-Womens Hospital of UPMC, Pittsburgh, PA

Background: Mammary myofibroblastoma (MFB), regarded as solitary fibrous tumor (SFT) by some in the past, is a tumor of the mammary stroma that typically express CD34, desmin and estrogen receptor. Recently, rearrangement or deletion of 13q14, resulting in loss of Rb expression has been described in MFBs. Since morphologic variants are relatively common in mammary MFB, establishing a correct diagnosis in a core biopsy can be challenging. Additionally, there are no clear guidelines regarding their management.

Design: Twenty-seven MFB cases were retrieved from our pathology database between 2002-2016. The clinical and pathologic findings were reviewed. Pathology slides were reviewed for classification of variants. Immunohistochemical (IHC) stains for Rb and STAT6 were performed in all available cases.

Results: There were 21 females (78%) and 6 males (22%) with a mean age of 56 years at diagnosis (range 31-91 years). Eleven cases (41%) presented with a painless palpable mass, the remaining were detected on imaging studies. Lesion size ranged from 0.5 to 10 cm (mean 1.9 cm). On ultrasound, the echogenicity of the lesions varied from hypoechoic to isoechoic to hyperechoic; most presented as well-circumscribed mass but 5 (19%) demonstrated indistinct margins. Fifty percent cases showed unusual morphology; 7 cellular, 5 lipomatous (50% or more fat), and 2 myxoid variants. The spindle cells were positive for CD34 (24 of 25, 96%, 19 diffuse and 5 focal), desmin (21 of 24, 88%), SMA (18 of 21, 86%) and ER (15 of 16, 94%). The Ki-67 was generally low including the cellular variant (1-5% in 11 of 15 cases, 6-10% in 2 cases, 11-15% in 2 cases). IHC for Rb showed negative staining in majority of cases (20 of 24 cases, 83%), although in some cases the interpretation of the staining was difficult due to many positive endothelial and inflammatory cells. None of the tumors showed positive staining with STAT6 (25 cases), S-100 (16 cases) or keratin (23 cases). Most lesions were excised, 4 had positive margins but no re-excision, none had local recurrence on average follow up of 36 months.

Conclusions: In our series, mammary MFBs are more common in females. The imaging features of MFB are nonspecific and can overlap with other lesions. Most MFBs show loss of expression of Rb protein and lack of nuclear expression for STAT6, suggesting MFB to be closely related to spindle cell lipoma than SFT. Routine excision of MFB is not necessary unless the tumor is rapidly growing or large.

216 Tumor Infiltrating Lymphocytes and PD-L1 Expression in Metastatic Breast Cancer

Jack Jacob[1], *Rachel Hazan*[2], *Joseph M Albanese*[3], *Susan Fineberg.* [1]Montefiore Medical Center, Bronx, NY, [2]Montefiore Medical Center, [3]Montefiore Medical Center, Bronx, NY

Background: Evidence of immune response, including high levels of tumor infiltrating lymphocytes (TILs), has been linked to improved prognosis in breast cancer (BC). The prognostic implications of TILs and expression of the immunoregulatory antigen PD-L1 in metastatic BC (mBC) however, are less well studied.

Design: We identified pts with BC and metastases and selected pts with slides and blocks from both the primary BC (pBC) and site of mBC . We evaluated stromal TILs in the primary tumor (pTILs) and the metastases (mTILs) using the guidelines proposed by the International Immuno-oncology Working Group. We reported an absolute stromal TILs % and also compared cases with and without >= 30% TILs. For PD-L1 immunohistochemistry (clone SP142) membrane expression on either tumor cells or lymphocytes was considered positive. Long term survival was defined as > 2 yrs from the date of metastasis.

Results: We identified 26 patients with matched pBC and mBC material. The average age of our pt population at presentation was 58.8 years. The receptor profiles of the pBCs were as follows: ER-/ HER2- (n=15), ER+ only (n=7), HER2+ only (n=2), ER+/ HER2+ (n=1), and ER+/HER2 equivocal n=(1). Sites of metastatic spread included CNS (8), liver (4), bone (4), lung and pleura (4), pericardium (2), GI tract (2), skin (1), and supraclavicular node (1). pTILs and mTILs averaged 12.6% (range: <1 - 40%) and 12.88% (range: 1 to 60%), respectively. Survival was > 2 yrs from date of mBC in 7 of 25 pts (28%). In comparing pBC to mBC, TILs increased in 12 cases , decreased in 11 cases and was unchanged in 3, with no correlation to survival (p-value: ns). mTILs were ≥ 30% in 5 cases, and 4 of these pts survived > 2 years after mBC dx, including one TNBC, one ER+, one HER2+ and one Her2 equiv case. TILs were <30% in 20 pts with evaluable survival, and only 3 survived > 2 yrs. When comparing mTILs ≥ 30% to survival > 2 yrs, a statistically significant correlation was noted (p= 0.0099). PD-L1 was evaluable in 24 cases with the following results: negative in both pBC and mBC (n=11), positive in pBC only (n=4), positive in mBC only (n=5), and positive in both pBC and mBC (n=4). When comparing survival to PD-L1 expression within the pBC or mBC, no statistically significant correlation was identified.

Conclusions: There is a statistically significant correlation between survival and elevated TILs in metastatic breast cancer sites. Our findings suggest that evidence of immune response in mBC is associated with improved survival.

217 Targeted Next Generation Sequencing Analysis of Metaplastic Breast Carcinoma

Zena Jameel[1], *Edi Brogi*[2], *Dara Ross*[3], *Britta Weigelt*[2], *Jorge Reis-Filho*[4], *Hannah Y Wen*[2]. [1]Wallington, NJ, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]New York, NY, [4]Memorial Sloan-Kettering CC, New York, NY

Background: Metaplastic breast carcinoma (MBC) is an aggressive subtype of invasive breast carcinoma, usually triple negative (TN), with poor response to systemic therapy. We reviewed targeted massively parallel next generation sequencing (NGS) data of MBC performed in our molecular diagnostic laboratory to identify potentially actionable alterations.

Design: We searched our database to identify MBC treated at our institution with NGS data available. The targeted NGS platform detects somatic mutations, copy number alterations (CNAs) and rearrangements in up to 468 cancer-related genes. Two pathologists reviewed all cases and confirmed the diagnosis. Receptor status was obtained from the pathology report.

Results: We identified 24 MBCs from 23 patients. One patient had two tumors tested. All but one MBC were high grade. Among high grade MBCs, the predominant metaplastic component in the primary MBC was matrix producing (n=12), squamous (n=5), spindle cell (n=3), and mixed (n=2). One patient had low-grade fibromatosis-like MBC. Receptor status was available for 18 MBCs, 12 were TN. Androgen receptor (AR) was tested in 12 cases, 2 were AR positive. NGS analysis of 14 primary and 10 metastatic tumors was performed. Among the 23 high grade MBC samples, the median number of somatic mutations in each case was 5 (range 0-9), the median number of somatic alterations (mutations and CNAs) was 9 (range 0-37). The most frequent mutations were *TP53* (20; 87%), and *PIK3CA* (6; 26%). Alterations affecting PI3K/AKT/mTOR pathway were seen in 8 (35%) MBCs. Mutation in *NF1* was found in 3 of 5 (60%) squamous MBCs but not in other histologic subtypes. The most frequent CNAs in high grade MBCs was MYC amplification (6; 26%), almost exclusively (5 of 6) in matrix-producing MBCs. Unlike high grade MBC, the low-grade fibromatosis-like MBC lacked *TP53* mutation. Mutations affecting *PIK3R1*, *TERT*, and *DROSHA* were detected in the metastatic sample available for analysis.

Conclusions: Metaplastic carcinoma has frequent mutations in *TP53* and PI3K/AKT/mTOR pathway. Whether MBC could benefit from PI3K/AKT/mTOR inhibitors warrants clinical investigation. Low-grade fibromatosis-like MBC is a rare low grade variant associated with indolent behavior. Genomic alterations in this subtype have not been fully investigated due to the rarity of the entity. The only low-grade fibromatosis-like MBC in this cohort developed distatnt metastasis and had *PIK3R1*, *TERT*, and *DROSHA* mutations, but lacks *TP53* mutation.

218 Prognostic Value of Prognostic Staging System Based on the 8th Edition of American Joint Committee on Cancer Staging Manual in Different Molecular Subtypes of Invasive Breast Cancer

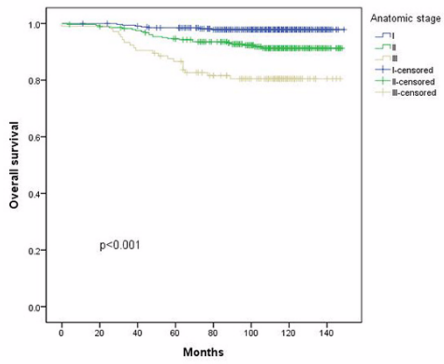
Nuri Jang[1], *Jung Eun Choi*[2], *Su Hwan Kang*[2], *Young Kyung Bae*[3]. [1]Yeungnam University Hospital, Daegu, [2]Yeungnam University College of Medicine, [3]Yeungnam University College of Medicine, Daegu

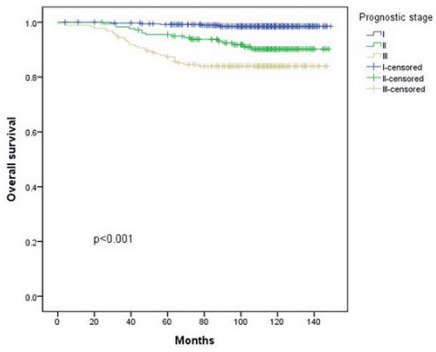
Background: The eighth edition of the American Joint Committee on Cancer (AJCC) staging manual incorporated biologic markers, such as tumor grade, estrogen receptor (ER) and progesterone receptor (PR) expression, human epidermal growth factor 2 (HER2) expression, and multigene assay, into the traditional anatomic staging systems (TNM classification) for a better prognostic classification of breast cancer (prognostic staging system). We aimed to investigate prognostic value of anatomic and prognostic staging system in different molecular subtypes of invasive breast cancer (IBC).

Design: A total of 725 IBC patients who received postoperative standard hormone therapy or chemotherapy were included for this study. All cases were restaged using the 8th AJCC anatomic and prognostic staging system. Molecular subtypes were defined using ER, PR, HER2 and Ki-67 index based on the 2013 St. Gallen International Expert Consensus.

Results: On prognostic staging, 445 (61.4%) were assigned to different stages (162, down-staged and 283, upstaged) compared with anatomic TNM stage. Both overall survival (OS) and disease-free survival (DFS) were significantly different in the different anatomic and prognostic stage groups (all p<0.001). The prognostic stage I and II patients restaged from anatomic stage I had significant differences in OS (p=0.001). However, there was no difference in OS between anatomic stage groups IA and IB (p=0.415). The different prognostic stage groups restaged from anatomic stage II also showed statistically significant differences in OS (p<0.001) and DFS (p=0.007). However, the restaged prognostic groups from anatomic stage III had no survival differences in either OS (p=0.360) or DFS (p=0.362). The prognostic stage groups showed significantly different OS and DFS in luminal A (p<0.001 and p=0.009), luminal B (HER2-negative) (p=0.001 ad p=0.028), luminal B (HER2-positive) (p=0.002 and p=0.012), and HER2-positive (p=0.027 and p=0.283) subtypes. However, prognostic groups in triple-negative subtype had no statistically significant differences in OS (p=0.420) and DFS (p=0.411).

Anatomic stage	Prognostic stage							Total
	IA	IB	IIA	IIB	IIIA	IIIB	IIIC	
IA	179	72	56	0	0	0	0	307
IB	16	10	3	0	0	0	0	29
IIA	0	79	37	25	61	0	0	202
IIB	0	5	18	21	12	10	16	82
IIIA	0	1	2	18	1	18	10	50
IIIC	0	0	0	0	4	19	32	55
Total	195	167	116	64	78	47	58	725





Conclusions: In conclusion, we observed that the prognostic staging system proposed in the 8th edition of AJCC refines the anatomic TNM staging system to predict survival for breast cancer.

219 Expression of p62 protein in invasive breast cancer and tumor microenvironment correlates with advanced clinicopathologic features

Israel Kasago[1], *Tony El Jabbour*[2], *Christine Sheehan*[3], *Ann Boguniewicz*[2]. [1]Albany Medical College, Albany, NY, [2]Albany Medical Center, Albany, NY, [3]Albany Medical College, Albany, NY

Background: p62 is a multifunctional protein with a regulatory role in many signaling cascades, including autophagy, a sequestration process that has been linked to therapeutic resistance in HER2 dependent breast cancer. Disruption of autophagy leads to an accumulation of p62, and recent evidence indicates that this defective clearance may contribute to a tumor-supporting microenvironment and promote tumorigenesis. In this study we evaluated p62 protein expression in invasive breast cancers, including the tumor microenvironment.

Design: Formalin-fixed, paraffin embedded sections from 112 invasive mammary carcinoma [85 ductal carcinomas (IDC) and 27 lobular carcinomas (ILC)]were immunostained by automated methods (Ventana Medical Systems, Inc, Tucson, AZ) using mouse monoclonal SQSTM1/p62 antibody (clone D5L7G, Cell Signaling). Cytoplasmic (Cp62) and nuclear (Np62) p62 immunoreactivity was semiquantitatively assessed in the tumor and adjacent benign component (when present) in all cases. Scoring was based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%). Presence of Cp62 and Np62 immunoreactivity was also assessed within the tumor microenvironment (Cp62tm and Np62tm, respectively). Results were correlated with clinicopathologic variables.

Results: Intense diffuse Cp62 was noted in 26/112 (23%) tumors and correlated with tumor grade (III>II>I, p=0.001) and HER2 status (HER2 36% pos vs 18% neg, p=0.036). Intense diffuse Np62 was noted in 15/112 (13%) tumors and correlated with menopausal age at diagnosis (30% pre vs 14% post vs 3% peri, p=0.016). Cp62tm was noted in 76/112 (68%) and Np62tm in 74/112 (66%) cases and correlated with advanced tumor stage (p=0.028, p=0.019), tumor size >2.0cm (p=0.014, p=0.004), and lymph node positive status (p=0.04, p=0.072 trend), respectively. Cp62tm showed a trend toward high tumor grade (p=0.064), while Np62tm showed a trend toward disease recurrence (p=0.08). On multivariate analysis, positive node status (p<0.0001) and disease recurrence (p<0.0001) independently predicted overall survival.

Conclusions: Overexpression of p62 was observed in tumors and in the tumor microenvironment and correlated with age at diagnosis and a more aggressive behavior overall. Further study of p62 protein expression and its potential role in breast cancer appears warranted.

220 Comparison of ß-Adrenergic Receptor Expression in De novo and Radiation-Associated Breast Angiosarcoma

Mohammad S Khan[1], *Bonnie Balzer*[2], *Fai Chung*[2], *Farnaz Dadmanesh*[3]. [1]Cedars Sinai Medical Center, Los Angeles, CA, [2]Cedars Sinai Medical Center, [3]Cedars-Sinai Medical Center

Background: Breast angiosarcoma (BAS) carries a dismal prognosis. Expression of ß-AR by immunohistochemistry (IHC) and therapeutic efficacy of ß-AR antagonists are described in angiosarcoma. Studies of ß-AR expression in BAS are limited. We studied ß2- and ß3-AR IHC expression in de-novo and radiation-associated (RA) BAS.

Design: 12 patients with BAS were selected: 6 with RA and 6 de-novo BAS including 1 patient with metachronous bilateral de novo BAS. 3 patients had a recurrence included in this series. 11 BAS were high grade and 1 was low grade. Slides from each case were reviewed and IHC for ß2- and ß3-AR was performed and scored as weak, moderate or strong based on cytoplasmic/membranous reactivity. Extent of expression based upon area was estimated by %.

Results: Expression of ß2-AR ranged from 65-100% and ß3-AR varied from 0-100% (Table 1). Expression was stronger for ß2-AR than for ß3-AR (Fig 1). Neither strength or extent of IHC correlated with de novo or RA status. Original and recurrent tumors were compared in 3 cases and showed no change.

Table 1. Results of IHC expression of B2-AR and B3-AR in BAS

Case	%/E B2-AR	%/E B3-AR	Status	Location	Site of recurrence
1	100/S	90/S	RA	R Breast Skin	
2	100/S	100/S	RA	R Breast	
3	90/S	0/NA	RA	L Breast	
4	100/S	90/Mo	RA	R Breast	
5	100/S	10/W	RA	R Breast	
	100/S	10/W			R Breast
6	65/Mo	80/Mo	RA	L Breast	
	80/Mo	60/Mo			L Chest wall
7	100/Mo	60/Mo	De-novo	R Breast	
8	70/Mo	5/W	De-novo	L Breast	
9	80/Mo	80/Mo	De-novo	R Breast	
10 (LG)	100/S	90/S	De-novo	L Breast	
11	90/S	70/Mo	De-novo	R Breast	
12*	100/S	70/Mo	De-novo	R Breast (B)	
	100/S	30/Mo		R Breast (M)	
	100/S	100/S			R Breast skin
13*	100/S	90/S	De-novo	L Breast (B)	
	100/S	100/S		L Breast (M)	

E: Extent; S: strong; Mo: moderate; W: weak; B: Biopsy; M: Mastectomy; NA: Not applicable; *The patient is the same in both cases.

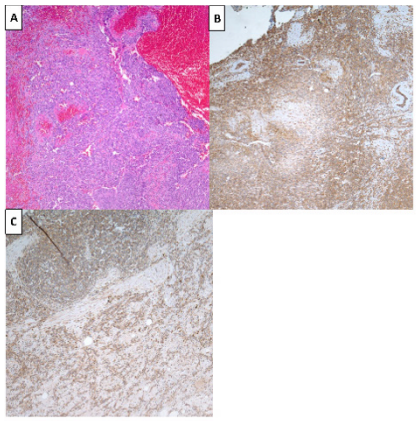


Fig 1- A. H & E of de-novo AS (Case 12); **B.** B2-AR immunohistochemistry highlighting the strong cytoplasmic and membranous staining within tumor cells; **C.** B3-AR immunohistochemistry highlighting moderate cytoplasmic and membranous staining within tumor cells.

Conclusions: Extent and strength of ß-AR IHC expression appear to correlate directly and ß2- tends to be stronger and more diffuse than ß3-AR expression. ß-AR IHC expression is not affected by de novo vs. RA status or primary vs. recurrent tumors. This small series is the first study comparing IHC ß-AR expression between de novo and RA BAS. Efficacy of ß-AR blockade has demonstrated dose dependency when used in combination treatment in AS. The potential utility of high levels of ß-AR expression in BAS as a predictor of therapeutic response is unknown. Additional studies are needed to determine whether ß-AR immunoreactivity can be used to monitor potential therapeutic response in selected cases.

221 A Nomogram for Predicting the Likelihood of Additional Axillary Nodal Metastases in Breast Cancer Patients with a Positive Intramammary Lymph Node

Thaer Khoury[1], *Rouzan G. Karabakhtsian*[2], *Anupma Nayak*[3], *Matthew G Hanna*[4], *Yisheng Fang*[5], *Xuan Peng*[6], *Li Yan*[6], *Xiaoxian Li*[7], *Oluwole Fadare*[8], *Christine Ambrosone*[6], *Simon D. Fung-Kee-Fung*[9], *Carmelo Gaudioso*[6], *Mohamed Mokhtar Desouki*[10]. [1]Buffalo, NY, [2]Montefiore Medical Center, Albert Einstein COM, Bronx, NY, [3]Perelman School of Medicine & Hospital of the University of Pennsylvania, Philadelphia, PA, [4]Memorial Sloan Kettering Cancer Center, New York, NY, [5]UT Southwestern Medical Center, [6]Roswell Park Cancer Institute, [7]Emory University, Atlanta, GA, [8]UC San Diego Health, San Diego, CA, [9]Roswell Park Cancer Institute, Buffalo, NY, [10]Vanderbilt University Medical Center, Nashville, TN

Background: There have been multiple nomograms to predict the likelihood of additional nodal metastases in breast cancer (BC) patients with a positive sentinel lymph node (SLN). The purpose of the study is to create a nomogram to predict additional axillary nodal metastases when a positive intramammary lymph node (intra-MLN) is encountered.

Design: A retrospective collaborative study among eleven academic institutions resulted in 113 BC candidate cases. The inclusion criteria were: at least one intra-MLN+; no neoadjuvant therapy; not stage 4; axillary lymph node dissection (ALD); known primary tumor size; known metastasis size; known extranodal extension (ENE) status (present/absent) in both the SLN and the intra-MLN; way of intra-MLN detection (clinical vs. pathologic); histologic subtype; Nottingham grade; biomarkers status; lymphovascular invasion (LVI); and focality (uni-/multi-). Univariate and multivariate logistic regression analyses identified factors predictive of positive ALD. Using these variables, a nomogram was constructed.

Results: On univariate analysis, the following variables were predictive of additional nodal metastases: number of intra-MLN/SLN- ($p<0.001$), ENE ($p=0.036$), histologic subtype ($p=0.034$), primary tumor size ($p=0.016$), way of intra-MLN detection ($p=0.051$) and LVI ($p<0.001$). In the multivariate analysis, intra-MLN/SLN- and LVI were statistically significant ($p<0.001$ and 0.001, respectively). The constructed nomogram including significant variables in the univariate analysis had area under the receiver operating characteristic (ROC) curve of 0.884

Conclusions: We have developed a nomogram that can be used to easily and accurately predict the likelihood of having residual disease in the axilla in patients with intra-MLN+ with or without SLN+.

222 The Clinical Significance of Metastatic Breast Carcinoma to Intra-Mammary Lymph Node

Thaer Khoury[1], *Matthew G Hanna*[2], *Anupma Nayak*[3], *Mohamed Mokhtar Desouki*[4], *Souzan Sanati*[5], *Yisheng Fang*[6], *Xiaoxian Li*[7], *Xuan Peng*[8], *Li Yan*[8], *Oluwole Fadare*[9], *Simon D. Fung-Kee-Fung*[10], *Carmelo Gaudioso*[8], *Rouzan G. Karabakhtsian*[11]. [1]Buffalo, NY, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]Perelman School of Medicine & Hospital of the University of Pennsylvania, Philadelphia, PA, [4]Vanderbilt University Medical Center, Nashville, TN, [5]Washington University, Saint Louis, MO, [6]UT Southwestern Medical Center, [7]Emory University, Atlanta, GA, [8]Roswell Park Cancer Institute, [9]UC San Diego Health, San Diego, CA, [10]Roswell Park Cancer Institute, Buffalo, NY, [11]Montefiore Medical Center, Albert Einstein COM, Bronx, NY

Background: While axillary lymph node status is considered significant prognostic factor, the significance of involved intramammary lymph node (intra-MLN) remains unclear.

Design: A total of 113 breast carcinoma cases with at least a single intra-MLN + were collected from 11 academic institutions. The exclusion criteria were: no subsequent axillary lymph node dissection (ALD) or status unknown, neoadjuvant therapy, stage IV status, T-stage unknown, size of nodal metastasis unknown unless one of the positive nodes (intra-MLN, sentinel lymph node, ALD) measured >2-mm (macrometastases), unknown biomarkers status, and/or no clinical follow up. AJCC N-stage was calculated twice, with and without intra-MLN. 5-year overall survival (OS) and relapse free survival (RFS) were calculated and correlated with the clinicopathologic variables. Multivariate survival model was constructed using TN-stage calculated with or without intra-MLN to investigate whether including intra-MLN in the TN-staging system can better predict the survival probability. Akaike information criterion (AIC) was used to evaluate the relative quality of statistical models.

Results: Excluding intra-MLN, there were 11 (9.7%), 57 (50.5%) and 45 (39.8%) stage, 1, 2 and 3, respectively. Including intra-MLN, there were 3 (2.7%), 51 (45.1%), and 59 (52.2%) stage 1, 2 and 3, respectively. Excluding intra-MLN, TN-stage correlated with OS ($p=0.016$) but not with RFS. Including intra-MLN, TN-stage correlated with OS ($p<0.001$) and RFS ($p=0.045$). The RFS was better predicted when intra-MLN was included in the staging with AIC of 162.98 compared to 166.21. The OS was better predicted when intra-MLN was included in the staging with AIC of 112.34 compared to 120.36

Conclusions: Intra-MLN is an adverse clinical factor. We recommend considering intra-MLN in the nodal staging. However, further studies with more cases and including cases with negative intra-MLNs are needed.

223 B7-H3 and B7-H4 Expression in Phyllodes Tumor of the Breast Detected with RNA In Situ Hybridization and Immunohistochemistry and Its Association with Clinicopathologic Features and T-cell Infiltration

Nah Ihm Kim[1], *Ji Shin Lee*[2]. [1]Chonnam National University Hwasun Hospital, [2]Chonnam National University Hwasun Hospital, Jeollanam-do

Background: Phyllodes tumors (PTs) of the breast are rare biphasic tumors with the potential for both local recurrence and distant metastasis. No convincing data are available to suggest any immunotherapeutic approaches. The aberrant expression of co-inhibitory B7 molecules, B7-H3 and B7-H4, in the tumor microenvironment has been attributed to reduced anti-tumor immunity and immune evasion, prompting the development of immunotherapeutic approaches. The present work was undertaken to evaluate the expression of B7-H3 and B7-H4 in PTs and its association with the grade and clinical behavior of PTs. In addition, the association of B7-H3 and B7-H4 expression with the T-cell infiltration was also assessed to investigate its roles in the regulation of tumor immune surveillance.

Design: B7-H3 and B7-H4 mRNA and protein expressions were determined by RNAscope in situ hybridization (ISH) and immunohistochemistry (IHC), respectively, in 101 PTs (60 benign, 26 borderline, and 15 malignant) using tissue microarray. Immunohistochemical staining for CD3 and CD8 was also performed.

Results: The staining patterns of B7-H3 and B7-H4 protein IHC were in concordance with the B7-H3 and B7-H4 mRNA ISH. B7-H3 mRNA and protein expression appeared to be concentrated mainly in the stromal compartment of PTs. However, B7-H4 mRNA and protein were negative in the stromal compartment of PTs. Stromal B7-H3 mRNA and protein positive expression was noted in 10 (16.7 %) and 31 (51.7 %) of 60 benign PTs, 12 (46.1 %) and 20 (76.9 %) of 26 borderline PTs, and 10 (66.7 %) and 13 (86.7 %) of 15 malignant PTs, respectively. Stromal B7-H3 mRNA and protein expression was increased as PTs progressed from a benign to borderline into the malignant grade (Pearson's R = 0.411, P = 0.000 and Pearson's R = 0.293, P = 0.003, respectively). The recurrence rate was higher in the stromal B7-H3 mRNA or protein-positive group than in the negative group but this difference was not significant. Stromal B7-H3 protein expression inversely correlated with the infiltration density of CD3+ and CD8+ T cells (P = 0.001 and P = 0.027, respectively).

Conclusions: These results suggest that B7-H3 is involved in the progression of PTs and B7-H3 may play a role in immune surveillance mechanisms of PTs.

224 Expression of Immune-Regulatory Proteins and Infiltration of Tumor Infiltrating Lymphocyte Subsets during In Situ to Invasive Transition of Breast cancer

Milim Kim[1], *Hyunjung Kim*[2], *Yul Ri Chung*[3], *So-yeon Park*[4]. [1]Seoul National University Bundang Hospital, Seongnam-si, Gyeonggi-do, [2]Seoul Medical University Bundang Hospital, Seongnam-si, Gyeonggi-do, [3]Seoul National University Bundang Hospital, [4]Seoul National Univ/Medicine, Gyeonggi-do, Korea

Background: Immune microenvironment is known to play a critical role in tumor progression and response to therapy. However, the role of immune-regulatory protein and tumor infiltrating lymphocytes (TILs) during in situ to invasive transition of breast cancer is poorly understood. This study was conducted to compare the expression of immuno-regulatory proteins and infiltration of TIL subsets in ductal carcinoma in situ (DCIS), microinvasive carcinoma and invasive carcinoma of the breast.

Design: A total of 578 cases comprising DCIS, microinvasive carcinoma and invasive carcinoma were selected and immunohistochemistry was performed for HLA-ABC, HLA-A, indoleamine 2,3-dioxygenase (IDO), CD4+, CD8+ and FOXP3+ T cells using tissue microarrays. TIL subset infiltration was manually counted in intratumoral and stromal areas.

Results: Expression of HLA-ABC, HLA-A, and IDO showed an association with positive hormone receptor status in DCIS, whereas their expression was associated with negative hormone receptor status in invasive carcinoma. Infiltration of TIL subsets was significantly higher in the hormone receptor-negative subgroup than in the hormone receptor-positive group in microinvasive carcinoma and invasive carcinoma, but not in DCIS. Expression of HLA-ABC, HLA-A and IDO was significantly lower in invasive carcinoma than in DCIS and microinvasive carcinoma, and their expression was not different between DCIS and microinvasive carcinoma in the whole

group and in the hormone receptor-positive subgroup. However, in the hormone receptor-negative subgroup, HLA-ABC and HLA-A expression was higher in microinvasvie carcinoma than in DCIS. As a whole, infiltration of CD4+, CD8+ and FOXP3+ TILs increased gradually during progression from DCIS to microinvasive and invasive carcinoma. While TIL subset infiltration was not different between DCIS and microinvasive carcinoma in the hormone receptor-positive subgroup, CD4+ and FOXP3+ TIL infiltration was significantly higher in microinvasive carcinoma than in DCIS within the hormone receptor-negative subgroup.

Conclusions: Our findings suggest that immune-regulatory protein expression and TIL subset infiltration play an important role in the progression of DCIS to invasive carcinoma but during different steps according to hormone receptor status.

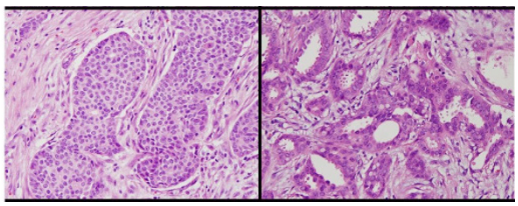
225 Clonality Analysis of Multifocal Ipsilateral Breast Carcinomas Using X-Chromosome Inactivation Patterns

Hayeon Kim[1], *Aeree Kim*[2], *Chung-Yeul Kim*[3]. [1]Korea University, Seoul, Seoul, [2]Korea University Guro Hospital, Seoul, [3]Korea University

Background: The definition of multifocal breast cancer is ambiguous and its incidence varies depending on the definition and detection methods. Multifocal breast cancers either have the same clonal origin or arise from completely distinct progenitor cells. The current American Joint Committee on Cancer Staging system and College of American Pathologists breast tumor guidelines state that only the largest tumor needs to be staged and studied immunohistochemically, on the assumption that they are of the same origin. However, this strategy has been criticized, because some multifocal tumors have been proven to have arisen from different clones.

Design: 71 cases of surgically resected multifocal breast cancers were selected. To detect and characterize the tumors of each clonal origin, a human androgen receptor gene (HUMARA) assay to compare the X-chromosome inactivation patterns of multiple tumors was conducted. The intrinsic subtypes according to immunohistochemical staining for the estrogen receptor, progesterone receptor, human epidermal growth factor, and Ki-67 were recorded.

Results: The intrinsic subtype was identical in 64 out of 71 (90.1%) cases, but seven (9.9%) cases had different intrinsic subtypes. 29 (40.8%) patients were revealed to be heterozygous for HUMARA. 64 patients (90.1%) had the same X chromosome inactivated in different tumors. Seven (9.9%) cases displayed different peaks in capillary gel electrophoresis, indicating that those tumors were not clonal in origin. All seven cases with different inactivated X chromosomes were invasive carcinomas of no special type. Five out of those seven cases showed almost identical or very similar histological features. Two cases did not display identical morphological features. Microscopic photos of a selective case is shown in Figure 1. Only one case showed a different intrinsic subtype based on immunohistochemistry.



Conclusions: In conclusion, this study showed that a proportion of ipsilateral multifocal breast cancers are new primary cancers, even though they have similar histological and immunohistochemical characteristics. In addition, tumors can be of different origin while they are in close proximity. These findings suggested that a precise understanding of the pathogenetic basis of multifocal breast cancers might be critical when formulating an optimal therapeutic strategy.

226 Differentially Expressed Genes in Matched Normal, Cancer, and Lymph Node Metastatic Tissues can Predict Clinical Outcomes in Patients with Breast Cancer

Nah Ihm Kim[1], *Ji Shin Lee*[2]. [1]Chonnam National University Hwasun Hospital, [2]Chonnam National University Hwasun Hospital, Jeollanam-do

Background: Genome-wide screening of transcriptome dysregulation among normal, cancer, and metastatic tissues would provide insights into the molecular basis of breast cancer (BC) metastasis.

Design: To identify differentially expressed genes (DEGs) in the metastatic progression of BC and to determine whether these genes have predictive power for clinical outcome, we profiled mRNA expression in matched normal, cancer, and lymph node metastatic tissues of seven patients with estrogen receptor-positive, HER2-negative BC by using massive parallel sequencing of RNA transcripts (RNA-seq).

Results: We totally detected 20,112 expressed genes. Using arbitrary cutoff criteria of |fold change| ≥ 2 and $p < 0.05$, we identified 1,522 and 664 DEGs between the normal and cancer tissues and between the cancer and nodal metastatic tissues, respectively. We identified 461 upregulated and 203 downregulated genes in nodal metastatic tissues compared with the corresponding cancer tissues. To better understand the function of DEGs, we conducted an enrichment analysis of KEGG pathway categorization for the dysregulated genes. The DEGs from the comparisons of normal vs. cancer tissues and cancer vs. nodal metastatic tissues were significantly clustered in one and eight KEGG pathways, respectively. The chemokine signaling pathway was the most significant pathway in the cancer to nodal metastasis transition (FDR = 2.15E−13). SQLE is one of the DEGs and encodes squalene epoxidase. We did SQLE immunohistochemical staining on tissue microarray sections of 198 BC cases. High SQLE expression was detected in 80 of 198 cases (40.4%) and was associated with worse disease-free and overall survival ($p = 0.001$ and $p = 0.001$, respectively) and independently predicted worse disease-free survival ($p = 0.043$) in patients with BC. Following BreastMark analysis, high SQLE mRNA expression in BC was significantly associated with poor prognosis in all group (HR = 1.467, p = 1.57E-10), lymph node negative group (HR = 1.781, p = 8.53E-08), lymph node positive group (HR = 1.337, $p = 0.011$), luminal A subtype (HR = 1.427, $p = 0.007$), and luminal B subtype (HR = 1.284, $p = 0.007$).

Conclusions: Using RNA-seq analysis in BC and their matching normal and lymph node metastatic tissues, we are able to identify DEGs associated with the metastatic progression of BC. The DEGs identified in this study can be used as new biomarkers for predicting the prognosis of patients with BC.

227 Negative Conversion of Progesterone Receptor Status after Primary Systemic Therapy is associated with Poor Clinical Outcome in Patients with Breast Cancer

Milim Kim[1], *Soomin Ahn*[2], *Hyunjung Kim*[3], *Yul Ri Chung*[2], *So-yeon Park*[4]. [1]Seoul National University Bundang Hospital, Seongnam-si, Gyeonggi-do, [2]Seoul National University Bundang Hospital, [3]Seoul Medical University Bundang Hospital, Seongnam-si, Gyeonggi-do, [4]Seoul Nat'l Univ/Medicine, Gyeonggi-do, Korea

Background: Alteration of biomarker status after primary systemic therapy (PST) is occasionally found in breast cancer. However, prognostic significance of such alteration remains unclear. This study was conducted to clarify the clinical implications of change of biomarker status in breast cancer patients treated with PST.

Design: The pre-chemotherapeutic biopsy and post-chemotherapeutic resection specimens of 442 breast cancer patients who had residual disease after PST were included in this study. The association between changes of biomarker status after PST and clinicopathologic features of tumors, and survival of the patients, were analyzed.

Results: Estrogen receptor (ER), progesterone receptor (PR) and HER2 status changed after PST in 18 (4.1%), 80 (18.1%), and 15 (3.4%) patients, respectively. ER and PR mainly underwent positive to negative conversion, whereas HER2 status underwent negative to positive conversion. Notably, alterations of biomarkers were more frequent in the luminal B subtype than in the luminal A, HER2+ and triple-negative subtypes. In survival analyses, negative conversion of ER and PR status after PST was associated with reduced disease-free survival. Moreover, a decline in the Allred score for PR in post-PST specimens was significantly associated with poor clinical outcome of the patients. HER2 change did not have prognostic significance. In multivariate analyses, negative PR status after PST was found to be an independent adverse prognostic factor in the whole patient group, in the adjuvant endocrine therapy-treated subgroup, and also in pre-PST PR positive subgroup.

Conclusions: ER and HER2 status changed little after PST, whereas PR status changed significantly. In particular, negative conversion of PR status was as a poor prognostic indicator, suggesting that re-evaluation of basic biomarkers is mandatory in breast cancer after PST for proper management and prognostication of patients.

228 Clinicopathological Significance of Triple-Negative Breast Cancer Subtypes Based on Immunohistochemical Surrogate Markers

Sewha Kim[1], *Sun Hee Sung*[2]. [1]CHA Bundang Medical Center, CHA University, Seongnam-si, Gyeonggi-do, [2]Ewha Womans University School of Medicine

Background: Triple-negative breast cancers (TNBC) are clinically challenging due to the disease heterogeneity of these lesions and there are no established therapeutic molecular targets. Recently, Burstein et al. identified four stable molecular subtypes of TNBC by mRNA profiling: luminal androgen receptor (LAR), mesenchymal (MES), basal-like immune-suppressive (BLIS), and basal-like immune-activated (BLIA). The purpose of this study was to classify the TNBC

subtypes using immunohistochemistry (IHC) surrogate panels by the Burstein's definition.

Design: IHC was performed to measure AR, GCDFP-15, claudin-3, E-cadherin, CK5/6, EGFR, IDO1, and FOXC1 levels using tissue microarrays constructed from 200 TNBC samples. Stromal tumor infiltrating lymphocyte (TIL) density was defined as the the ratio of the area occupied by mononuclear inflammatory cells to the total intratumoral stromal area. Ki-67 labeling index (LI) was measured using digital image analysis software.

Results: The 200 TNBCs were classified as LAR type (AR+ and/or GCDFP-15+, n = 22, 11.0%), MES type (claudin 3– and/or E-cadherin–, n = 23, 11.5%), basal-like type (CK5/6+ and/or EGFR+, n = 85, 42.5%), mixed type (tumors with features of two or three types, n = 60, 30%), and null type (tumors with none of the above features, n = 10, 5%). Basal-like type TNBCs were further categorized as BLIA type (IDO1+ and FOXC1-, n = 27) or BLIS type (IDO1- and FOXC1+, n = 11). LAR type was significantly associated with a younger patient age, apocrine histological features, the lowest stromal TIL density, and the lowest Ki-67 LI. MES type was significantly associated with tumor cell discohesiveness and metaplastic features. Basal-like type was significantly associated with a younger patient age, high histologic grade, the highest stromal TIL density, the highest Ki-67 LI, and a p53 mutation staining pattern. Large tumor size, nodal metastasis, low stromal TIL density, and BLIS type were independent predictors of worse clinical outcome according to both univariate and multivariate analyses.

Conclusions: This study suggests that the surrogate IHC panel may define TNBC subtypes with distinct clinicopathological characteristics. BLIS type was a more distinct fatal subset within the aggressive TNBC tumors.

229 Predicting DCIS Recurrence Risk Using a Machine Learning-Based High-Content Image Analysis Approach

Sergey Klimov[1], *Islam MILIGY*[2], *Arkadiusz Gertych*[3], *Yi Jiang*[4], *Padmashree Rida*[5], *Ian Ellis*[6], *Emad Rakha*[7], *Ritu Aneja*[5]. [1]Roswell, GA, [2]University of Nottingham, [3]Cedars-Sinai Medical Center, West Hollywood, CA, [4]Georgia State University, [5]Georgia State University, Atlanta, GA, [6]City Hospital, NHS Trust, Nottingham, England, [7]University of Nottingham, Nottingham, England

Background: The incidence of ductal carcinoma-in-situ (DCIS), a pre-invasive lesion, has drastically escalated over the past few decades. Breast conserving surgery is the most common treatment for DCIS; however, up to 30% of patients ultimately suffer recurrences. This study aimed to provide an accurate machine learning (ML)-based recurrence classifier by extracting and combining image features obtained from full slide image analysis.

Design: High-resolution images of a clinically-annotated set of 286 H&E-stained pure DCIS lumpectomy surgical samples were analyzed through a twofold classification pipeline. First, a sliding window extracted 166 texture features from hematoxylin areas of the slide that were used to annotate slide regions into functional domains (stroma, normal duct, cancer duct, lymphocyte region, and blood vessels) through a ML classifier. Next, over 3,000 texture distribution and spatial features in the annotated slides were compared between patients who later experienced recurrence, versus those that did not. An additional ML classifier was then trained and optimized using full slide features, to predict future recurrence risk.

Results: Both classification steps in the pipeline produced high accuracies for texture classification (99%) and patient recurrence (80%). Using only 20 texture features, the optimized and cross-validated model was able to very significantly stratify patients into high-risk [with 10-year disease-free survival (DFS) of 48%] and low-risk (10-year DFS of 85%) of recurrence subgroups, through survival analysis (HR=4.2, p<0.001). This model retained significance after correcting for necrosis, size, grade, and margins, and vastly outperformed them (HR=4.65, p<0.001).

Conclusions: Our novel texture-based classification system provides a high degree of confidence in recurrence risk prediction for DCIS patients, with vastly superior patient stratification compared to any current clinicopathological variable. The richness of high content data allowed discovery of image features that may be hidden to even a trained eye. Thus, our full slide classification system can accurately identify patients who will likely need heightened surveillance and can be candidates for additional therapy.

230 Genomic Profiling of Metaplastic Carcinoma and Associated in Situ Carcinoma of the Breast

Gregor Krings[1], *Yunn-Yi Chen*[2]. [1]UCSF, San Francisco, CA, [2]Univ. of California, San Francisco, San Francisco, CA

Background: Metaplastic carcinomas of the breast (MC) are rare tumors with differentiation of the neoplastic epithelium into squamous or mesenchymal-like elements. Most MC are triple negative (TNBC) but are heterogeneous in histologic subtype and gene expression. Outcomes and chemotherapy responses are worse than other TNBC and vary by histologic subtype. Genetics of MC may help explain these differences but are not well characterized. Progression and clonality of MC and associated in situ carcinoma (DCIS) are also poorly defined. We profiled MC and DCIS using capture-based next generation sequencing (NGS) and compared the results among MC subtypes and to TCGA TNBC.

Design: DNA was extracted from 28 MC and matched normal tissue, including paired DCIS in 8 MC and paired non-metaplastic ductal carcinoma (IDC) in 2 MC. NGS was performed targeting exons of 480 cancer genes and *TERT* promoter. Single nucleotide variants, insertions/deletions and copy number alterations (CNA) were evaluated.

Results: Hotspot (hs) *PIK3CA*/*PIK3R1* mutations were identified in 61% MC (vs 14% TCGA TNBC, p<.001), including 89% non-chondroid (NCh) and 0% chondroid (Ch) MC. *PIK3R1* mutations were enriched in MC (25% vs 2% TCGA TNBC, p<.001). One of only 2 NCh MC with wildtype *PIK3CA*/*PIK3R1* had an activating *AKT1* mutation. Hs *KRAS* or *HRAS* mutations were identified in 18% MC (vs 1% TCGA TNBC, p=.004), including 21% NCh and 0% Ch tumors. *TERT* promoter mutations (*TERT*p) were present in 37% NCh and 0% Ch MC. No (0%) pure spindle cell carcinomas (SpC) had *TP53* mutations, compared to 78% non-SpC (p=.003; p=.02 vs TCGA TNBC). No *CDK4*/*MDM2* co-amplifications were seen in osseous (Oss) or other MC. Squamous cell carcinomas (SCC), Ch, and Oss MC were genomically unstable and SpC/mixed SpC-SCC were genomically more stable. DCIS (2 SpC, 2 Ch, 3 SCC, 1 SpC-SCC) was clonally related to paired MC, with additional pathogenic mutations in MC of 6 cases. Clonal analysis of 2 MC (SCC, Ch) showed stepwise progression from DCIS to IDC to MC.

	PIK3CA/PIK3R1 %	**KRAS/HRAS** %	**TERT**p %	**TP53** %	CNA (mean; range)
MC (n=28)/TCGA TNBC (p)	61/14 (<.001)	18/1 (.004)	25/-	64/58 (.671)	-
SpC, pure (n=5)	100	20	80	0	3; 0-7
SpC-SCC (n=5)	100	20	40	60	1; 0-6
SCC (n=6)	83	40	17	67	15; 0-20
Ch (n=9)	0	0	0	89	23; 8-43
Oss (n=3)	67	0	0	100	24; 23-25

CHONDROID	SQUAMOUS	SQUAMOUS-SPINDLE	SPINDLE
Lack activating PI-3 kinase or Ras pathway mutations	Enriched in activating PI-3 kinase and Ras pathway mutations		
No TERT promoter mutations	Frequent TERT promoter mutations		
Frequent TP53 mutations	Frequent TP53 mutations		No TP53 mutations
Genomically unstable		Genomically stable	

Conclusions: MC are a genetically distinct subgroup of TNBC, but mutation profiles and CNA vary according to histologic subtype. NCh MC are enriched in *PIK3CA*/*PIK3R1* and *KRAS*/*HRAS* mutations, whereas Ch MC lack these features. SpC, SCC and SpC-SCC but not Ch MC have *TERT*p. In contrast to other MC variants and most TNBC, SpC lacked *TP53* mutations in this study. SpC and SpC-SCC are genomically stable, whereas Ch, SCC and Oss MCB are unstable. MC can arise from stepwise progression of DCIS through an IDC intermediary.

231 Molecular Characterization of Mammary Pleomorphic Adenomas

Gregor Krings[1], *Gregory Bean*[2], *Bradley Stohr*[2], *Elizabeth Hosfield*[3], *Sandra J Shin*[4], *Yunn-Yi Chen*[5]. [1]UCSF, San Francisco, CA, [2]UCSF, San Francisco, CA, [3]Kaiser Permanente, Oakland, CA, [4]Albany Medical College, Albany, NY, [5]Univ. of California, San Francisco, San Francisco, CA

Background: Mammary pleomorphic adenomas (MPA) are morphologically similar to salivary gland pleomorphic adenomas (SGPA), benign tumors with *PLAG1* or *HMGA2* rearrangements. MPA are usually benign but may recur or rarely be associated with

carcinoma. Distinction from metaplastic carcinoma with chondroid differentiation (MC) may be challenging, especially on core biopsy. Some consider MPA to be at the low end of a spectrum of matrix-producing tumors including MC, but molecular characterization is lacking. We analyzed MPA by capture-based next generation sequencing (NGS), fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) and compared the results to MC and SGPA to gain insight into the biology of these rare lesions and help with differential diagnosis(Ddx).

Design: 8 MPA, 15 SGPA and 11 MC were studied. IHC was performed for SOX10, HMGA2, CK5/6, ER, p53 and Ki67. FISH was performed with *PLAG1* and *HMGA2* split-apart probes. DNA was extracted from 4 MPA, 11 MC and matched normal tissue for NGS. NGS targeted exons of 480 cancer genes and 40 introns. Single nucleotide variants, insertions/deletions and copy number alterations (CNA) were evaluated.

Results: *PLAG1* FISH was positive in 1/8 (13%) MPA compared to 6/13 (46%) SGPA and 0/8 (0%) MC (p=ns). All MPA and MC (n=8 each) were *HMGA2* FISH-, and 1/14 (6%) SGPA had *HMGA2* amplification. All SGPA were HMGA2 IHC+ (n=15), compared to 2/8 MPA and 2/11 MC (p<.001 each). Of 2 HMGA2 IHC+ MPA, 1 had focal complex copy loss involving distal *HMGA2*. This MPA had truncating *KMT2A* and *ARID5B* mutations, and another MPA had inactivating *ARID1A* mutation; truncating *KMT2A* and *ARID1A* mutations were also identified in MC (1/11 each). *TP53* was mutated in 10/11 (91%) MC and 0/4 (0%) MPA (p=.001). Most (7/11) MC but no (0/4) MPA had additional pathogenic aberrations, which included recurrently mutated *RB1* (2/11), *FGFR1* (2/11) and *NOTCH1* (2/14). By IHC, p53 was negative or diffuse positive in 10/11 (91%) MC, which correlated with mutation type, compared to 0/6 (0%) MPA (p=.001). Mean Ki67 index was higher in MC (48%) than MPA (1.2%; p=.001). MC had more CNA (mean 23, range 9-43) than MPA (mean 2.2, range 0-5; p=.002).

Immunohistochemical and FISH Features of MPA, SGPA, and MC

	PLAG1 FISH%	**HMGA2** FISH%	HMGA2 IHC% (n)	ER% (n)	CK5/6% (n)	SOX10% (n)	p53 IHC negative or strong diffuse + (n)	Ki67 mean%; range (n)
MPA	13 (8)	0 (8)	25 (8)	50 (6)	100 (5)	88 (8)	0 (6)	1.2; <1-2 (6)
SGPA	46 (13)	7 (14)	100 (15)	20 (15)	100 (15)	100 (15)	0 (15)	2.7; <1-7 (15)
MC	0 (8)	0 (8)	18 (11)	0 (11)	91 (11)	100 (11)	91 (10)	48; 10-80 (10)

FOR TABLE DATA, SEE PAGE 121, FIG. 231

Conclusions: MPA are neoplasms with clonal genomic aberrations and share features with SGPA, including *PLAG1* rearrangement. Chromatin remodeling genes are implicated in MPA pathogenesis, which overlaps with MC. In contrast to MC, MPA lack *TP53* mutations and are genomically stable. *TP53* status and p53/Ki67 IHC may be useful in the Ddx of MPA with MC.

232 Folate Receptor- alpha (FR α), Glycoprotein Nonmetastatic Melanoma B (GPNMB), BRCA1 Associated Protein (BAP1) and β-Catenin Are Highly Expressed in Triple Negative Breast Carcinoma

Uma Krishnamurti[1], *Jing Yang*[2], *Limin Peng*[2], *Cynthia Cohen*[3], *Xiaoxian Li*[1]. [1]Emory University, Atlanta, GA, [2]Emory University, [3]Emory Univ, Atlanta, GA

Background: Therapies targeting Folate receptor alpha (FRα) in ovarian cancer and glycoprotein nonmetastatic melanoma B (GPNMB), a transmembrane glycoprotein, in melanoma and breast cancer are in clinical trials. BRCA1 associated protein (BAP1), a nuclear protein, encoded by a tumor suppressor gene, is a deubiquitinating enzyme and loss of its expression has been shown to correlate with adverse clinicopathologic features in renal cell carcinoma. Overexpression or aberrant expression of β-catenin, a subunit of the cadherin protein complex, is associated with many cancers. The expression of these four proteins in triple negative breast carcinoma (TNBC) are not well studied.

Design: Immunohistochemistry (IHC) for FR α, GPNMB, BAP-1, and β-catenin was performed on tissue microarrays composed of 78-83 TNBC cases with duplet 1.0 mm cores from each case. Staining score (H-score) was calculated as a percentage of positive cells x staining intensity (1-3) and correlated with tumor grade, stage, lymph node metastasis (LNM), overall survival (OS) and disease-free survival (DFS). Cytoplasmic staining of FRα and GPNMB, membranous staining of β-catenin, and nuclear staining of BAP1 were scored. No aberrant staining of FRα, GPNMB, or β-catenin was observed. H-score > median is defined as high H-score.

Results: Forty-four of 83 (53%) cases were positive for FRα, 71 of 81(88%) for GPNMB, 62 of 78 (79%) cases for β-catenin, and 75 of 79 (95%) for BAP1. The median ($25^{th\ quantile}$, $75^{th\ quantile}$) H-scores of FRα, GPNMB, and β-catenin were 5 (0, 210), 140 (30, 225), and 225 (10, 285) respectively. BAP1 is a nuclear stain with median ($25^{th\ quantile}$, $75^{th\ quantile}$) H-score of 210 (120, 270). As nuclear H-score decreased, there was increased cytoplasmic staining. Complete loss of nuclear BAP1 (score 0) was only seen in 4/79 (5% cases). The H-scores for FRα and GPNMB were not associated with tumor grade, stage, LNM, OS and DFS. High H-score (>225) for β-catenin was associated with higher tumor stage (stage II+III vs I) [OR (95% CI) =2.75(1.08, 7.00), p=0.0344] but not other parameters.

Conclusions: High β-catenin H-score is associated with higher stage TNBC. High expression of FRα and GPNMB in TNBC might provide an opportunity for targeted therapy. The significance of aberrant high cytoplasmic BAP1 staining needs to be further investigated.

233 Molecular Profiles of Bone Metastases in Breast Cancer

Melissa Krystel-Whittemore[1], *Jochen Lennerz*[2], *G. Petur Nielsen*[3], *Elena Brachtel*[3]. [1]Massachusetts General Hospital, Boston, MA, [2]Massachusetts General Hospital and Harvard Medical, Boston, MA, [3]Massachusetts General Hospital, Boston, MA

Background: Molecular alterations in metastatic breast cancer are important for prognosis and targeted treatments. PIK3CA is the most commonly mutated gene in estrogen receptor (ER) positive breast cancer, and associated with resistance to hormonal treatment. TP53 is the most frequent mutation in ER negative breast cancer, with worse prognosis than TP53 wildtype. Mutational profiles have been studied in primary and metastatic breast cancer, but are challenging in bone metastases due to decalcification processes.

Design: Seventy-four bone samples from 66 patients with metastatic breast cancer were evaluated for oncogenic mutations with NGS SNAPSHOT using the Illumina next generation sequencing (NGS) platform, reported as single nucleotide variations (SNVs) or insertions/deletions (indels). ER and PR immunohistochemistry (IHC) was performed in all primary breast carcinomas (n=66) and most bone metastases (n=62). HER2 testing was performed by IHC on primary breast (n=62) and bone samples (n=55), complemented by fluorescence in-situ hybridization (FISH) in 43 cases.

Results: Patient age at breast cancer diagnosis was 51 years on average (range 23-70) with recurrences at 56 years after a mean interval of 79 months. 9 patients (14%) had bone metastasis as initial manifestation. 14 patients died of disease (10 ER+, 4 ER-). Most frequently, patients had grade 2/3 ER+ HER2- breast cancers (n=57, 86%) with lymph node involvement (N1-3, n=40). Mean (median) tumor size was 2.8 (2.5) cm. 4 patients were HER2+ (6%), and 4 (6%) triple negative. NGS SNAPSHOT showed 55 oncogenic mutations (50 SNVs, 5 indels) in 38 patients. 18 patients had no reported mutations and 10 failed due to insufficient DNA. Most frequent mutations were PIK3CA SNVs (n=24, 43%) and TP53 (n=13; 10 SNVs, 3 indels, 23%). Less frequent mutations included FBXW7, APC, PIK3R1, FGFR2, HRAS, and ATM (each 1 case). 8 of 14 patients (57%) who died of disease had TP53 mutations. [Table 1].

Table 1.

Mutation	Frequency	ER+	HER2+	Triple negative
PIK3CA	24	24	1	0
TP53	13	9	1	4
SMAD4	3	2	1	1
ESR1	3	3	1	0
CDH1	2	2	0	0
BRCA2	2	2	0	0
MET	2	2	0	0

Conclusions: This study characterizes a unique cohort of bone metastases in breast cancer that has previously not been examined by molecular methods. The most common mutation in bone metastases was PIK3CA, all in ER+ patients. TP53 was the second most frequent mutation, the most common mutation in patients with ER negative disease, and the most common mutation in patients who died of disease. Additional mutations were identified in which further investigation could yield potential therapeutic targets for metastatic disease.

234 P53 Signature in Non-Malignant Breast Tissue in Patients with Li-Fraumeni Associated Breast Cancer

Maria G Kuba[1], *Susan Lester*[2], *Judy Garber*[3], *Deborah Dillon*[2]. [1]Brigham and Women's Hospital, Boston, MA, [2]Brigham & Women's Hospital, Boston, MA, [3]Dana Farber Cancer Institute

Background: Li-Fraumeni syndrome (LFS) is an autosomal dominant hereditary cancer syndrome in which breast cancer is the most common malignancy among women. Germline TP53 mutation is the hallmark of this syndrome. In this study, we examine TP53 protein expression by immunohistochemistry in non-malignant breast tissue in LFS patients with breast cancer in an attempt to understand very early events in the development of this disease.

Design: Immunohistochemistry for TP53 (clone DO-7, dilution 1:500, DAKO; EnVision detection system) was performed in 25 cases of

LFS with at least 1 block with normal tissue adjacent to tumor and/or 1 separate block with normal tissue only. Twenty-seven reduction mammoplasty cases were used as controls (one block per case). Using a modification of the "p53 signature" definition in the fallopian tube (Lee Y et al, *J Pathol* 2007), cases were classified as TP53 positive if at least 12 consecutive nuclei in adjacent acini in a lobule showed moderate to strong expression. The number of foci (TP53-positive lobules) was calculated per block examined.

Results: Eleven of 25 (44%) LFS cases had detectable TP53 signatures, whereas the 27 controls were all negative ($p<0.001$). Four control cases were considered high risk based on family history of breast cancer (n=3) or personal history of Peutz-Jeghers syndrome (n=1). None of the high-risk controls showed a TP53 signature. Among LFS cases, 2/11 positive cases showed nuclear enlargement in the TP53 positive epithelium, but this was not diagnostic of FEA, ADH or DCIS. The presence of TP53 positive patches was unrelated to the type of germline mutation present.

Conclusions: TP53 positive patches (TP53 signatures) in non-malignant breast tissue are common in patients with LFS-associated breast cancers and not seen in controls. The TP53 signature may be even more common than detected here if additional blocks are studied. These TP53 patches may constitute non-morphologic high-risk cancer precursor lesions.

235 Hyperechoic Lesions of the Breast Diagnosed on Core Needle Biopsy: Types and Incidence of Lesions and Radiologic-Pathologic Correlation

Maria G Kuba[1], *Catherine S Giess*[1], *Tad Wieczorek*[2], *Susan Lester*[3]. [1]Brigham and Women's Hospital, Boston, MA, [2]Brigham and Women's Hospital, Boston, MA, [3]Brigham & Women's Hospital, Boston, MA

Background: Ultrasound (US) uses high-frequency sound waves to interrogate breast tissue, and features such as margins, shape, and orientation are used in predicting the likelihood of malignancy and guiding management. The internal echotexture of lesions relative to fat is a less predictive feature. However, most lesions hyperechoic relative to adipose tissue are benign and rarely undergo biopsy. Because rare malignant hyperechoic breast lesions have been reported, core needle biopsy (CNB) is sometimes warranted.

Design: Pathology reports of CNBs performed between 1/1/04 and 7/31/17 were searched for the words "breast" and "echogenic" or "hyperechoic". Radiologic and pathology reports were reviewed.

Results: A total of 123 cases were identified, comprising <1% of all CNBs. The indications for CNB included interval change, high risk of breast cancer (due to personal or family history of breast cancer or other high risk breast lesion), patient request, no mammographic correlate, or pregnant/post-partum patient with palpable mass.

Only 14 of the lesions were uniformly hyperechoic and all but 1 of these lesions had circumscribed margins. All were lesions with a component of adipose tissue or fat content: 7 angiolipomas, 4 lactational adenomas, 1 fat necrosis, and 2 vascular lesions (1 with atypical features). Twelve lesions were hyperechoic but had some areas that were hypoechoic. Only 3 had circumscribed margins. Half of the diagnoses were of lesions that contain adipose tissue: 4 fat necrosis, 1 myofibroblastoma, 1 panniculitis. The other diagnoses were a variety of benign stromal and epithelial lesions. The remaining 97 lesions had areas of mixed echogenicity. Although some of the diagnoses were of adipose containing lesions (e.g. fat necrosis, lactational adenoma, hamartoma, myofibroblastoma), many were not. In contrast to the hyperechoic lesions, 27 of the 97 mixed echogenicity lesions or 28% were malignant (22 invasive carcinomas, 3 lymphomas, and 2 metastases).

Conclusions: Breast lesions with hyperechoic echotexture on US are unusual and comprise a very small minority of masses undergoing biopsy. The pathologist should be aware that such lesions often correlate with a component of adipose tissue. The majority of predominantly hyperechoic lesions are benign. Although rare cases of malignancies presenting as a hyperechoic lesion have been reported, none were seen in this series. Lesions with mixed echogenicity were more frequently malignant.

236 Predictors Of Pathologic Response To Trastuzumab-Based Neoadjuvant Treatment In A Consecutive Monoinstitutional Series Of Breast Carcinomas Tested With Frontline Dual-Color HER2 FISH

Elisabetta Kuhn[1], *Alessandra Bisagni*[2], *Ione Tamagnini*[2], *Cristina Bassano*[2], *Elisa Gasparini*[2], *Roberto Di Cicilia*[2], *Giuseppe Falco*[2], *Guglielmo Ferrari*[2], *Foroni Moira*[2], *Giorgio Gardini*[2], *Giancarlo Bisagni*[2], *Moira Ragazzi*[3]. [1]Arcispedale S. Maria Nuova-IRCCS, Reggio Emilia, Italy, Reggio Emilia, Italy, [2]Arcispedale Santa Maria Nuova - IRCCS, [3]Arcispedale S. Maria Nuova-IRCCS, Reggio Emilia, Italy

Background: The pathological response to trastuzumab-based neoadjuvant therapy (TNT) has an important prognostic role, but is extremely variable among patients and still not fully understood. Although previous studies have supported the correlation between TNT response and both *HER2* amplification and immunohistochemistry (IHC), as yet reliable predictive factors of TNT response are missing. This study aims to investigate the contribution of different clinico-pathological features to TNT pathologic response in a series of *HER2*-positive breast cancers (BCs) based on frontline dual-color *HER2* FISH.

Design: 100 consecutive BC patients treated in our institution with TNT from 2009 to 2016 were included in this study. Pre-treatment biopsies and post-treatment surgical specimens were collected and analyzed by *HER2* FISH and IHC following the 2013 ASCO/CAP guidelines. Residual tumor, in both breast and lymph node specimens, was quantified and tumor regression was scored according to Pinder's histopathological criteria. Clinico-pathological features were recorded. Statistical analyses were performed and a p-value <0.05 was considered statistically significant.

Results: Among the 100 BCs, *HER2* amplification level was low in 23 cases, intermediate in 16 cases, and high (HA) in 61 cases, whereas HER2 IHC scored 0 in 7 cases, 1+ in 9, 2+ in 16 and, 3+ in 68. Strong correlation between HA and 2+/3+ IHC score was found ($p<0.0001$). TNT achieved pathological complete response (pCR) in 27 of 100 BCs (Table). Patients with pCR had significantly lower PR levels ($p=0.0248$) and a higher *HER2*/CEP17 ratio ($p=0.0298$). pCR correlated significantly with HA ($p=0.04$) and IHC 2/3+ ($p=0.005$). In 73 patients without pCR, we evaluated pathological features in paired pre-treatment and post-treatment specimens. In post-treatment samples, when compared with pre-treatment biopsies, we found a significant reduction of both ki67 proliferation index ($p<0.0001$) and *HER2* amplification level ($p<0.0001$). Notably, 9 of 61 (15%) BCs became *HER2* FISH negative after treatment. Regression score correlated with HA ($p=0.0006$), but not with IHC.

Table. Comparison between clinico-pathological features in pre-surgical biopsy of breast cancer patients with and without complete pathological response to trastuzumab-based neoadjuvant therapy

FEATURE	no pCR	pCR	p-value
Total	73	27	
Mean Age, ys (range)	56 (31-84)	57 (29-81)	n.s.*
Mean Clinical size, mm (range)	36 (10-80)	37 (10-70)	n.s.*
Clinical STAGE, n I-II III-IV	64 52 (81.3%) 12 (18.7%)	24 18 (75.0%) 6 (25.0%)	n.s.^
Histotype Ductal Lobular	69 (94.5%) 4 (5.5%)	27 (100.0%) 0 (0%)	n.s.^
GRADE 1 2 3	0 (0%) 40 (54.8%) 33 (45.2%)	0 (0%) 10 (37.0%) 17 (63.0%)	n.s.^
ER range (mean)	0-100% (52.0)	0-100% (33.0)	n.s.*
ER <1% ≥1%	25 (34.2%) 48 (65.8%)	10 (37.0%) 17 (63.0%)	n.s.^
PGR range (mean)	0-100% (27.0)	0-90% (7.3)	0.0248*
PGR <20% ≥20%	43 (58.9%) 30 (41.1%)	25 (92.6%) 2 (7.4%)	0.0013^
Ki67 range (mean)	5-90% (39.0)	10-80% (40.0)	n.s.*
Ki67 <20% ≥20%	6 (8.2%) 67 (91.8%)	1 (3.7%) 26 (96.3%)	n.s.^
HER2 FISH amplification level Low/Intermediate High	33 (45.2%) 40 (54.8%)	6 (22.2%) 21 (77.8%)	0.0406^
HER2 immunohistochemistry 0/1+ 2+/3+	16 (21.9%) 57 (78.1%)	0 (0%) 27 (100.0%)	0.0051^

total, number of cases; ER, estrogen receptor; PGR, progesterone receptor; n.s., no significant; *Mann-Whitney test; ^Fisher's test.

Conclusions: Based on our findings, pCR correlated with lower PR levels, other than HA and IHC 3+. Moreover, HA but not IHC correlated with minimal residual disease. Interstingly, the reduction of *HER2* amplification level along with negativity in a relevant percentage of post-treatment residual BCs suggest a role of tumor heterogeneity in the incomplete response to TNT.

237 GATA3 is a Positive Prognostic Marker in Invasive Breast Cancer Patients, Especially With Less Aggressive Disease

Elisabetta Kuhn[1], *Massimo Pedriali*[2], *Rosa Rinaldi*[2], *Paola Secchiero*[3], *Patrizia Querzoli*[4]. [1]University of Ferrara, Ferrara, [2]University of Ferrara, Ferrara, Italy, [3]University of Ferrara, Ferrara, Italy, [4]Università degli Studi di Ferrara

Background: GATA binding protein 3 (GATA3) has emerged as a sensitive immunohistochemical marker for breast carcinoma (BC). Interestingly, GATA3 expression is positively correlated with estrogen receptor (ER) expression. Moreover, some studies have investigated GATA3 as a prognostic marker in BC patients, with conflicting findings. Thus, we undertook the current study to evaluate the expression of GATA3 by immunohistochemistry in a large series of BCs with long follow-up and its prognostic value.

Design: A total of 702 consecutive primary invasive BCs were diagnosed and resected between 1989 and 1993 in our institution. All BCs were arranged in tissue microarrays, immunostained for ER, GATA3, ki-67, p53 and, progesterone receptor (PGR) and, scored as the percentage of positive BC cells using a computer-aided image analyzer (Eureka Interface System, Menarini). HER2 was scored according to ASCO/CAP 2013 guidelines. Clinico-pathological data (including patient age, tumor histology, pathologic stage, grading, and follow-up data) were retrospectively collected. Statistical analyses with a p-value <0.05 were considered significant.

Results: GATA3 was evaluable in 608 (87%) of 702 cases and was positive (≥1%) in 413 (68%) cases and negative (<1%) in 195 (32%) cases, with a GATA3 median score of 50% (range 0%-100%). GATA3 positivity correlated significantly with lower grade (p<0.0001), pT1 (p=0.0463) and stage I (p=0.0049). Among the biological factors, GATA3 expression correlated with ER+ (p<0.0001), PR+ (p<0.0001), wild-type p53 immunohistochemical pattern (p<0.0001) and HER2 negativity (p=0.0373). In our patients with a median follow-up of 181 months, GATA3 positivity correlated significantly with a better overall survival (p=0.0008). Moreover, GATA3 expression correlated with a better overall survival in patients with less aggressive BCs: Elston and Ellis grade 1 and grade 2 (p=0.0473 and p=0.0177); pT1 (p=0.0026); pN0 (p=0.0062); stage I (p=0.0431); stage II (p=0.0107); ER+ (p=0.0184); PR+ (p=0.0146); ki-67<20% (p=0.0177); HER2- (p=0.0005); and in BC with wild-type p53 immunohistochemical pattern (p=0.002).

Conclusions: Our findings indicate that GATA3 is a positive prognostic marker in BC patients, especially in patients with biologically less aggressive BCs.

238 Comparison of AJCC Anatomic and Prognostic Stage Groups in Breast Cancer: Analysis of 3126 Cases from a Single Institution

Ashish Kurundkar[1], *Jacob P Britt*[2], *Gene Siegal*[3], *Shi Wei*[4]. [1]University of Alabama at Birmingham, Birmingham, AL, [2]University of Alabama at Birmingham, [3]Univ. of Alabama at Birmingham, Birmingham, AL, [4]UAB, Birmingham, AL

Background: Prognostic factors in breast cancer (BC) largely include histologic grade (G), stage, ER, PR and HER2 status. The AJCC anatomic stage groups (ASG) take into account primary tumor (T), regional lymph node (N) and distant metastasis (M) and are arguably most powerful in predicting survival outcomes in BC. The 8th edition of AJCC has now consolidated the aforementioned factors into new prognostic stage groups (PSG). Further, recent studies have identified a number of multigene panels with substantial power in predicting BC recurrence. To that end, the Oncotype Dx score has been incorporated into PSG in the subset of BC with a T1-2/N0/M0/G1-3/HER2-/ER+/PR± profile. This study was aimed at comparing the discriminating power of ASG versus PSG.

Design: All BC cases diagnosed at the authors' institution were identified between 1998 and 2013. The clinicopathologic parameters of the primary BC along with outcomes achieved were recorded. Analysis of distant relapse-free survival (RFS) was performed using the Kaplan-Meier method and the log-rank test.

Results: Of the 6143 BCs diagnosed in the study period, 3126 had all the elements required for PSG and thus were included in the study. The median follow-up was 4.4 years. Compared to ASG, the application of PSG assigned 29.5% and 22% of cases to higher and lower stage groups, respectively. 11% of cases changed more than one stage group from ASG. In 110 (3.5%) cases, PSG were undetermined due to the lack of a place among the 10 anatomic and biomarker categories outlined in the stage group table, of which the most common profiles were T1/N0/M0/G2/HER2-/ER+/PR- (39), T3/N0/M0/G1-3/HER2-/ER+/PR+ (36), and T2/N1/M0/G2-3/HER2-/ER+/PR+ (23). The subset of BCs with a T1-2/N0/M0/G1-3/HER2-/ER+/PR± profile for which Oncotype Dx was not performed were assigned based on the anatomic and biomarker categories regardless of other multigene signature scores. PDG provided an improved overall discriminating power in predicting RFS compared to ASG (^{2}=422, P<.0001 vs. ^{2}=334.8, P<.0001). This improvement was significant in ASG I (^{2}=8.1, P=0.02) but more dramatically seen in ASG II (^{2}=56.1, P<.0001) and ASG III (^{2}=45.2, P<.0001).

Conclusions: The new AJCC PSG provided a superior yet imperfect tool in predicting BC survival outcomes. Further refinement of this table to provide additional categories and to incorporate other multigene panels (such as MammaPrint, PAM50 or Breast Cancer Index) may be necessary in the pursuit of precision medicine.

239 Detection of Residual Breast Cancer in Breast Excisions by the LUM Imaging System

Conor R Lanahan[1], *Michele A Gadd*[1], *Michelle C Specht*[1], *Rong Tang*[1], *Thomas Bischof*[2], *Barbara L Smith*[3], *Elena Brachtel*[4]. [1]Massachusetts General Hospital and Harvard Medical School, Boston, MA, [2]Lumicell, Inc., [3]Massachusetts General Hospital, Boston, MA, [4]Massachusetts General Hospital, Boston, MA

Disclosures: Thomas Bischof: *Employee*, Lumicell, Inc.

Background: Obtaining tumor-free margins is critical for local control of breast cancer. Currently, 20-40% of patients undergoing breast conserving surgery require surgical re-excision for carcinoma at or close to surgical margins identified on permanent sections. Frozen section analysis of breast margin tissue is not feasible in most institutions. The LUM Imaging System (Lumicell, Inc., Wellesley, MA) is an integrated imaging platform consisting of LUM015 fluorescent agent that is injected preoperatively, a hand-held imaging device, and software to detect areas of increased fluorescent uptake in the surgical cavity.

Design: With IRB approval and informed consent, LUM015 was administered to 45 patients undergoing breast conserving surgery for biopsy-confirmed breast cancer. 271 tissue samples including intraoperative lumpectomy cavity walls and final shaved margins were evaluated with the LUM Imaging System (mean 6 specimen parts per patient, range 1-11). All tissues underwent routine histopathology processing and diagnosis. We correlated imaging findings with histopathological results as a gold standard for determining the accuracy of the technology.

Results: 34 patients had invasive carcinoma with mean tumor size of 1.2 cm (range 0.1-3.5 cm) with or without ductal carcinoma in situ (DCIS), 8 had DCIS only, and 3 had no residual carcinoma found in the main lumpectomy specimen. 6 patients had grade 1 tumors, 19 patients had grade 2 tumors and 17 patients had grade 3 invasive carcinomas. The majority of carcinomas (n=40) were estrogen receptor (ER) positive, with one HER2 positive case. 60% (6 of 10) of patients with positive margins (3 invasive, 3 DCIS) had grade 3 carcinomas, and 90% (9 of 10) were ER positive. On a per-tissue basis, the LUM Imaging System correctly identified tumor at the tissue surface with 100% sensitivity (6/6 tissues) and 66% specificity (141/214 tissues) (Table 1). The device correctly identified 6 positive cavity margins intraoperatively (2 invasive and 4 DCIS).

LUM Imaging per tissue – Histology margin evaluation	
True Positive	6
False Positive	73
False Negative	0
True Negative	141
Sensitivity	100%
Specificity	66%

Conclusions: Use of the LUM Imaging System did not affect routine histopathology processing and diagnosis. The LUM Imaging System enabled surgeons to visualize, identify and remove residual invasive carcinoma as well as DCIS in the breast excision cavity during surgery. Further clinical trials are in progress to study this technology in a larger patient cohort.

240 Clonal Evolution in the Progression from Ductal Carcinoma In Situ To Invasive Ductal Carcinoma

Ju Youn Lee[1], *Rui Bi*[2], *Felipe Geyer*[2], *Hannah Y Wen*[3], *Raymond S Lim*[1], *James Hicks*[4], *Britta Weigelt*[3], *Jorge Reis-Filho*[5]. [1]MSKCC, [2]Memorial Sloan-Kettering Cancer Center, New York, NY, [3]Memorial Sloan Kettering Cancer Center, New York, NY, [4]USC, [5]Memorial Sloan-

Kettering CC, New York, NY

Background: Ductal carcinoma *in situ* (DCIS) is a *bona fide* non-obligate precursor of breast cancer, and may harbor intra-lesion genetic heterogeneity. We have previously provided direct evidence of clonal selection in the progression to invasive ductal carcinoma (IDC) by single cell sequencing copy number analysis. Here, we sought to define the genetic heterogeneity of DCIS and whether subclones harboring distinct mutations are selected in the progression from DCIS to IDC.

Design: Fourteen cases comprising synchronous DCIS and IDC were retrieved and subjected to central review and microdissection of each component separately. DNA samples of DCIS (n=16) and IDC (n=15) and matched normal DNA were subjected to whole-exome sequencing (WES; n=27) or massively parallel sequencing targeting all coding regions of cancer-related genes (n=4). Somatic genetic alterations and mutational signatures were defined using validated bioinformatics algorithms. Clonal decomposition analysis was performed using a Bayesian clustering model (PyClone) to infer the clonal and subclonal populations and determine the clonal shifts in the progression from DCIS to IDC.

Results: Somatic mutation and copy number analysis of DCIS samples revealed recurrent genetic alterations affecting genes significantly mutated in breast cancer, including *TP53*, *PIK3CA* and *GATA3* mutations and *CCND1* amplification. In all patients, the synchronous DCIS and IDC samples were found to be clonally related. In 3 cases, a minor DCIS subclone likely constituted the substrate for the development of the IDC, suggesting clonal selection. In 10 cases, sufficient somatic mutations were detected to infer the mutational signatures present in truncal and branch mutations found in DCIS samples and their matched IDC. In nine of these cases, the aging signature (i.e. signature 1) was the dominant signature in both sets of mutations, whereas in the remaining case that occurred in a *BRCA1* germline mutation carrier, truncal and branch mutations were enriched for the mutational signature 3, which associated with defective homologous recombination DNA repair. These findings suggest that the mutational processes appear not change substantially in the progression from DCIS to IDC.

Conclusions: Our findings corroborate the concept that DCIS is a direct non-obligate precursor of breast cancer, that intra-lesion genetic heterogeneity may be present in pre-invasive lesions, and that progression to invasive breast cancer occur through a variety of evolutionary processes.

241 Histologic Characterization of Invasive Breast Carcinoma Classified as Normal-Like by PAM50 Assay

Eleanor Lewin[1], *Yujing Jan Heng*[2], *Michael Pyle*[3], *Rulla Tamimi*[4], *Laura Collins*[2], *Gabrielle Baker*[2]. [1]Beth Israel Deaconess Medical Center, Boston, MA, [2]Beth Israel Deaconess Medical Center, Boston, MA, [3]Beth Israel Deaconess Medical Center, [4]Harvard T.H. Chan School of Public Health

Background: Gene expression profiling has defined five major intrinsic subtypes of primary invasive breast cancer (BrCa): Luminal (Lum) A, Lum B, HER2-enriched (HER2-E), basal-like (BL), and normal-like (NL). The morphologic features of these subtypes have been variably well-characterized; however, the histologic features of NL BrCa have only been evaluated in a small number of cases (n=24, Heng 2017). The validity of the NL subtype has been questioned, with some publications suggesting that NL BrCa may be an artifact of tissue procurement or may signify the presence of abundant normal epithelial or stromal cells. The purpose of this study was to characterize the histologic features of BrCa categorized as NL.

Design: In a dataset of 845 BrCa from the Nurses' Health Study, 71 (8.4%) were categorized as NL via the PAM50 assay. Whole slide images from 44 of these invasive BrCa cases (>1mm) were available for review. We evaluated a variety of histologic features including tumor type, grade, and cellularity; available biomarker status was obtained.

Results: Of the 44 available BrCa classified as NL, 21 were ductal (IDC), 9 were lobular (ILC), 8 had ductal and lobular features (ICDL), and 6 were mucinous. 15 cases (34%) were grade 1, 24 (55%) were grade 2, and 5 (11%) were grade 3. Overall, 72.7% (32/44) demonstrated minimal (<10%) tubule formation. Nine cases (20%) exhibited high tumor cellularity (>50%), 24 cases (55%) had moderate cellularity (10%-50%), and 11 (25%) had low cellularity (<10%). 78% were ER+, 20% were HER2+, and 4% were triple-negative.

Conclusions: The NL BrCa evaluated in this study displayed a spectrum of morphologic features; however, ILC and ICDL, which are characterized by minimal tubule formation, are relatively over-represented in this cohort (39%). Specifically, ILC comprises 21% of this series, contrasting with a rate of ~ 5% in BrCa overall (WHO 2012). By comparison, data from The Cancer Genome Atlas (TCGA) classified 24 of 850 (2.8%) BrCa as NL; of these, 10 (41.7%) were ILC and 20/24 (83%) had minimal tubule formation (Heng, 2017), similarly suggesting that the lobular phenotype may preferentially cluster in this molecular subtype. The presence of at least moderate cellularity in most (75%) of our NL BrCa argues against the hypothesis that this subtype reflects low tumor cellularity or contamination by normal breast tissue. This study provides histologic characterization of the largest series of NL BrCa to date and encourages further investigation.

242 HER2/neu "Triple-Equivocal" Breast Cancer: A Clinicopathologic Analysis of 32 Cases with An Emphasis on its Frequency and Potential Associations

Leo Lin[1], *Somaye Zare*[2], *Abrar G Alghamdi*[3], *Andres Roma*[4], *Farnaz Hasteh*[5], *Marie Dell'Aquila*[1], *Oluwole Fadare*[2]. [1]UC San Diego Health, San Diego, CA, [2]UC San Diego Health, San Diego, CA, [3]University of California, San Diego, San Diego, CA, [4]University of California San Diego, San Diego, CA, [5]Univ. of California, San Diego, San Diego, CA

Background: A subset of breast cancers are found to give equivocal results by both immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). Recomendations from ASCO/CAP allow for the use of an alternate probe for cases that give equivocal results using the HER2/CEP17 dual FISH probe. However, some cases remain equivocal even with the use of an alternate probe. At our institution, HER2/neu is performed by both FISH and IHC on all newly diagnosed cases of primary and metastatic breast cancer, which presented an opportunity to define the frequency and analyse the clinicopathologic features of the small subset of breast cancers that are "triple equivocal" for HER2/neu: equivocal by IHC, equivocal by FISH with a primary HER2/CEP17 dual probe, and equivocal by FISH with an alternate control probe (HER2/LIS1).

Design: HER2/neu results for 1201 consecutive cases of breast cancer were assessed, and we defined the frequency of each potential combination of IHC, FISH (primary dual probe: HER2/CEP17) and FISH (an alternate dual probe: HER2/LIS1, used for all cases with equivocal results with HER2/CEP17). Triple equivocal cases were compared to other subsets regarding patient age, tumor grade, histotype, ER and PR status, AJCC/TNM stage, and rate of pathologically confirmed distant metastases. All HER2/neu results were rendered using 2013 ASCO/CAP criteria

Results: There were 32 triple-equivocal cases, representing 2.67% of 1201 consecutive breast cancers. Compared to cases that were HER2/neu amplified using a dual HER2/CEP17 probe, triple equivocal cases were more frequently positive for ER (72% vs 94%, p=0.0091) and PR (60% vs 78%, p=0.049), and showed a higher frequency of pathologically-confirmed distant metastases (15% vs 31%, p=0.024) on univariate analyses. Triple-equivocal cases also showed a higher frequency of distant metastases compared to HER2/neu non-amplified cases using the dual HER2/CEP17 probe (31% vs 5%, p<0.0001) . However, triple-equivocal cases showed no significant differences from the aforementioned 2 groups regarding patient age, tumor grade, histotype, and stage distribution. Furthermore, they showed no statistically significant differences from cases that became HER2/neu-amplified with the HER2/LIS1 dual probe regarding any of the aforementioned parameters

Conclusions: HER2/neu triple equivocal breast cancer constitutes less than 3% of cases. Significant clinicopathologic associations were noted for this distinctive subset, as outlined above, and require further study

243 Frequency of DNA Mismatch Repair Deficiency in Breast Cancers Using a Large Tissue Microarray Cohort of Breast Cancer Cases Including Triple Negative Breast Cancers

Ying-Chun Lo[1], *Marguerite Pinto*[2], *Malini Harigopal*[3]. [1]Yale School of Medicine, North Haven, CT, [2]Yale University, New Haven, CT, [3]Yale Univ/Medicine, New Haven, CT

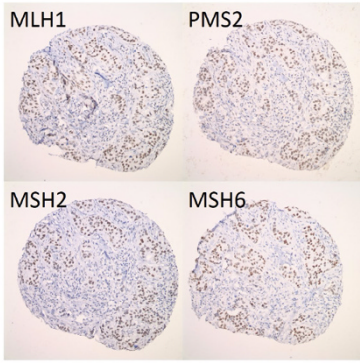
Background: Pembrolizumab was approved by the FDA in early 2017 for patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or DNA mismatch repair deficient (dMMR) solid tumors that have progressed after prior treatment. Loss of expression of DNA mismatch proteins can occur by (1) promoter hypermethylation as seen in sporadic cancer and (2) germline mutations seen in hereditary cases. Immunohistochemistry (IHC) identifies tumors with dMMR for the 4 main DNA MMR proteins (MLH1, PMS2, MSH2, MSH6) with near 100% specificity. MMR protein IHC is considered a surrogate of PCR based-MSI testing. Frequency of dMMR has been reported in many cancers including colorectal, endometrial, stomach, non-small cell lung cancer and esophageal ranging from 10% to 73% in single DNA MMR protein. Few studies showed an approximately 2% frequency of loss of 1 of the 2 pairs in breast carcinomas (BC). This study reports our series of BC with dMMR by IHC.

Design: MLH1, PMS2, MSH2 and MSH6 IHC were done using tissue microarrays (TMA). Three TMA's were constructed from 280 invasive BC which included various histologic subtypes (ductal, lobular, mixed ductal and lobular, mucinous and micropapillary). A fourth TMA was made from triple negative (TN) BC of African American patients.

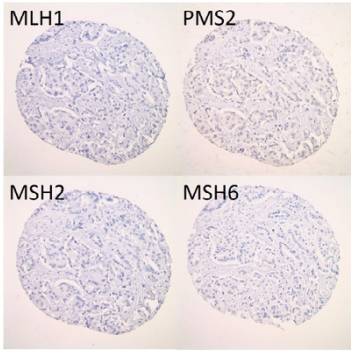
Nuclear IHC staining for the 4 MMR proteins (MLH1, PMS2, MSH2 and MSH6) were assessed. MLH1 loss is associated PMS2 loss; but if PMS2 is lost MLH1 may persist. Similarly, MSH2 loss is associated with MSH6 loss. Loss of expression was reported only if all tumor cells stained negative for each antibody. Normal breast tissue in the TMA served as positive internal controls.

Results: Of 385 BC cases: Loss of expression of at least 1 mismatch protein was noted in 22 /280 (7.9%) of all and 11 /105 (10.4%) TN BC (Table 1). Loss of expression of at least 2 of the 4 mismatch repair proteins was present in 20 (7.1%) of all and 8 (7.6%) TN BC. Loss of expression of at least 3 of the 4 mismatch repair proteins was found in 9 (3.2%) of all and 5 (4.8%) TN BC. Four (1.4%) of all and 2 (1.9%) TN BC lost the expression of all 4 mismatch repair proteins. Among the 4 mismatch repair proteins, MSH2 had the highest frequency of deficiency (all: 7.1%, TN: 8.5%), followed by MSH6 and PMS2, while MLH1 had the lowest frequency of deficiency (all: 1.8%, TN: 2.9%).

dMMR	All BC	TN
At least 1	22	11
At least 2	20	8
At least 3	9	5
All 4	4	2
MLH1	5	3
PMS2	11	6
MSH2	20	9
MSH6	18	8
Total cases	280	105



Example of MMR retention



Example of MMR deficiency

Conclusions: Frequency of dMMR in all BC is roughly 8%, regardless of histologic type. Trend towards more frequent dMMR in TN BC compared to all BC. MSH2 deficiency is most common in our study.

244 Utility of GATA3, PAX8 and WT1 Immunohistochemistry in Distinguishing Metastatic Ovarian Serous Carcinoma from Breast Primary Using Tissue Microarrays

Ying-Chun Lo[1], *Tao Zuo*[2], *Haris Mirza*[3], *Padmini A Manrai*[4], *Natalia Buza*[5], *Marguerite Pinto*[6], *Malini Harigopal*[7]. [1]Yale School of Medicine, North Haven, CT, [2]Boston, MA, [3]Yale School of Medicine, New Haven, CT, [4]Yale University/Yale New Haven Health, New Haven, CT, [5]Yale University, New Haven, CT, [6]Yale University, New Haven, CT, [7]Yale Univ/Medicine, New Haven, CT

Background: Metastatic carcinomas to the breast constitute less than 3% of all breast malignancies. In the majority of cases the patient has a known clinical history of malignancy. Histologic comparison to the primary may be helpful but in difficult cases immunohistochemical (IHC) stains are valuable. Distinguishing metastases from a breast primary is crucial for clinical management purposes. In this study we investigated metastatic ovarian carcinomas to the breast in a large cohort of breast and ovarian carcinomas using tissue microarrays (TMA). Both breast carcinoma (BC) and epithelial ovarian cancer are CK7+ and CK20-. In this study we assessed the value of GATA3, PAX8 and WT1 IHC in distinguishing ovarian metastases from a breast primary.

Design: We assessed GATA3 (L50-823), PAX8 (rabbit polyclonal) and WT1 (6F-H2) IHC expression using BC and ovarian serous carcinoma TMA's. WT1 was interpreted as positive only if there was nuclear staining. Cell lines and normal tissue in the TMA's served as positive and negative internal controls.

Results: Ninety-nine BC and 226 ovarian serous carcinoma cases were studied. Forty-eight (48.5%) of 99 BC were ER-, including 32 (32.3%) triple negative cases. Sixty-five of 99 (65.7%) BC showed positive GATA3 expression. Only 22 of 48 (45.8%) ER- BC and 13 of 32 (40.6%) triple negative BC were GATA3+, while 43 of 51 (84.3%) ER- BC were GATA3+. One (1.0%) BC stained for PAX8 and none stained for WT1 (0% nuclear, however 10.1% cytoplasmic). Four of 226 (1.8%) ovarian serous carcinomas showed some GATA3 staining while 211 (93.4%) showed positive PAX8 staining and 100 (44.2%) showed some WT1 staining. Though GATA3 has been known more commonly to be lost in ER- BC, it still demonstrates good overall sensitivity (65.7%). PAX8 shows much higher sensitivity for ovarian carcinomas compared to WT1 (93.4% vs. 44.2%). When combining PAX8 and WT1, the sensitivity for ovarian carcinomas is increased to 96.9%. GATA3 shows great specificity for BC (95.1%) while PAX8 and WT1 show even greater specificity for ovarian carcinomas (99.0% and 100%, respectively) in the context of this study.

IHC	Breast Carcinoma	Ovarian Serous Carcinoma
GATA3	65	4
PAX8	1	211
WT1	0	100
PAX8 + WT1	1	219
Total cases	99	226

Conclusions: In our TMA study using a large cohort of breast and ovarian serous carcinomas we found that PAX8 (93.4% vs 1.0%) and WT1 (44.2% vs 0%) are sensitive and exceedingly specific stains (in combination 96.9% vs. 1.0%) for distinguishing ovarian from a breast primary. GATA3 is specific in distinguishing breast primary from a metastatic ovarian serous carcinoma (65.7% vs. 1.8%).

245 Higher Level of VEGF mRNA is Significantly Associated with Poorer Outcome in Triple Negative Breast Cancer

Shihleone Loong[1], *Joe Yeong*[2], *Jeffrey Chun Tatt Lim*[3], *Aye Aye Thike*[4], *Puay Hoon Tan*[5]. [1]Singapore General Hospitl, [2]Singapore General Hospital, Singapore, [3]Singapore General Hospital, Singapore, [4]Singapore General Hospital, [5]Singapore

Background: Triple negative (TN) breast cancers have aggressive behavior, early relapse and lack targeted therapy options. The Chemokine receptor 4 (CXCR4) plays a role in chemotaxis and organ-specific metastasis while vascular endothelial growth factor (VEGF) is a critical mediator of angiogenesis. We investigated mRNA levels and protein expression of VEGF and CXCR4 in a series of TN breast cancers, correlating with clinicopathological parameters and outcome.

Design: The cohort comprised 663 TN breast cancers diagnosed at Singapore General Hospital. Immunohistochemistry was performed using antibodies to basal markers (CK14, 34 E12), EGFR, VEGF and CXCR4 on sections cut from tissue microarray blocks. Positive biomarker expression was defined as staining of 10% or more carcinoma cells displaying membrane staining for CXCR4 and cytoplasmic staining for VEGF. Among these, 182 tumors were subjected to VEGF and CXCR4 mRNA profiling using NanoString Technologies. Disease free (DFS), metastasis free (MFS) and overall (OS) survivals were correlated with mRNA levels and protein expression.

Results: VEGF and CXCR4 protein expression was observed in 41% and 61% of tumors respectively. Histologic grade was associated with both VEGF (p=0.031) and CXCR4 (p=0.001) protein expression. A tri-panel of CK14, EGFR, and 34 E12 determined 86% of TN breast cancers in this series to be basal-like. These basal-like tumors were rich in VEGF protein expression (p=0.005). Among 182 cases which

underwent mRNA assays, 101 (55%) tumors showed high VEGF mRNA expression while 93 (51%) tumors harbored high CXCR4 expression using median cut-off values. The follow-up ranged from 2 to 236 months (mean 85, median 71 months). Recurrences and metastases occurred in 184 (28%) and 134 (20%) of patients respectively. Protein expression of both VEGF and CXCR4 was not associated with outcomes. However, women whose tumors harbored high mRNA level of VEGF disclosed poorer DFS, MFS and OS (p=0.007, p=0.0008 and p=0.037 respectively). On multivariate analysis, high level of VEGF mRNA was an independent predictor of DFS (HR 2.247, 95%CI 1.027-4.918, p=0.043) and MFS (HR 0.042, 95%CI 1.026-4.091, p=0.042).

Conclusions: Our study demonstrates that protein expression of VEGF and CXCR4 in TN breast cancer is significantly associated with adverse characteristics. Higher VEGF mRNA expression is a predictor of recurrence and metastasis. Upregulationof VEGF in this disease may be of prognostic valueand provide new insights for further therapeutic paradigms.

246 Solid Papillary Carcinomas with Reverse Polarity (Solid Papillary Breast Carcinomas Resembling the Tall Cell Variant of Papillary Thyroid Neoplasms) Harbor Recurrent Mutations Affecting IDH2: A Validation Study

John Lozada[1], *Felipe Geyer*[2], *Thais B de Oliveira*[1], *Barbara Alemar*[1], *Fresia Pareja*[3], *Patrizia Querzoli*[4], *Gabor Cserni*[5], *Maria Foschini*[6], *Emad Rakha*[7], *Edi Brogi*[8], *Britta Weigelt*[8], *Jorge Reis-Filho*[9]. [1]MSKCC, [2]Memorial Sloan-Kettering Cancer Center, New York, NY, [3]MSKCC, New York, NY, [4]Università degli Studi di Ferrara, [5]Bacs-Kiskun County Teaching Hospital, Kecskemet, [6]University of Bologna, Bologna, Italy, [7]University of Nottingham, Nottingham, England, [8]Memorial Sloan Kettering Cancer Center, New York, NY, [9]Memorial Sloan-Kettering CC, New York, NY

Background: Solid papillary carcinoma with reverse polarity (SPCRP), also known as solid papillary breast carcinoma resembling the tall cell variant of papillary thyroid neoplasms, is a rare breast cancer subtype with morphology resembling that of the tall cell variant of thyroid papillary carcinoma. We have previously identified highly recurrent hotspot mutations affecting *IDH2* R172 or *TET2* in conjunction with PI3K pathway-related genes as likely drivers of SPCRP. Here we sought to characterize their unique histology and to investigate the frequency of *IDH2* mutations in an independent series of SPCRPs.

Design: Five SPCRPs not previously subjected to molecular analyses were centrally reviewed by three breast pathologists. Estrogen receptor, progesterone receptor and HER2 status was retrieved from the pathology reports. Tumor DNA samples from each case were extracted from microdissected representative histologic sections, and subjected to Sanger sequencing for the *IDH2* R172 hotspot locus.

Results: The histology and genetic features were consistent across the 5 SPCRPs included in this study. They were all characterized by solid and papillary architecture with circumscribed and invasive tumor nodules composed of a double layer of epithelial cells with reverse polarity. Colloid-like secretion was found in three cases, and four cases displayed sclerotic stroma. The nuclei displayed grade 2 atypia, occasional grooves and intranuclear cytoplasmic inclusions. All cases were of histologic grade 1 (tubule formation 2 + nuclear grade 2 + mitotic rate 1) and of triple-negative phenotype. Single nucleotide variants affecting the *IDH2* R172 hotspot residue were identified in all five SPCRPs studied (3 R172S, 1 R172T and 1 R172G).

Conclusions: We identified *IDH2* R172 hotspot mutations in 100% of *bona fide* SPCRPs tested, validating the high frequency of mutations affecting this gene, as well as the genotypic-phenotypic correlation previously reported in this rare breast cancer type. Additional sequencing analyses to ascertain the presence of concurrent mutations in PI3K pathway-related genes is in progress, and the results will be available at the meeting.

247 Immunohistochemical subtype of breast cancer among Lao Women: A study in limited resource setting of Lao PDR

Thitsamay Luangxay[1], *Siriphone Virachith*[2]. [1]Mittaphab Hospital, Vientiane, Vientiane, [2]Tokyo Medical and Dental University

Background: The recent increasing number of Lao women with breast cancer urge the need of investment for diagnosis and treatment. Histopathological examination should be the minimum requirement to diagnose breast cancer. This should include the immunohistochemical testing for estrogen receptor (ER), progesterone receptor (PR) and HER-2 receptor. Currently, none of the related data had been reported among these women. These missing information posed difficulties to predict the prognosis and responsiveness to the treatment. The lack of infrastructure of health care facilities in Lao PDR is the reason why these three main treatment markers are not routinely tested.

Design: The formalin-fixed and paraffin embedded tissue blocks of 76 cases with primary breast cancer were retrieved starting from 2013 to 2016. Patients' information and previous histological reports were reviewed. Immunohistochemistry was done using antibodies against estrogen receptor (ER), progesterone receptor (PR), Human epidermal growth factor 2 (HER-2/neu) and Ki-67.

The purpose of this study is to evaluate the prevalence of the immunohistochemical subtypes of breast cancer among Lao women by using immunohistochemistry (according to St Gallen 2017 guideline) and to study their correlation to their clinicopathological features in order to help guide for better decision of treatment plan and prognosis of patients.

Results: The mean age of the patient was 49 years with major histologic type of invasive ductal carcinoma NOS (90.7%). The proportion of each subtype showed hormone receptor-positive and HER-2-negative:36.8%, hormone receptor-positive and HER-2-positive:2.6%, hormone receptor-negative and HER-2-positive:10.5% and triple negative:42.1%. Estrogen receptor were positve in 39.4% while progesterone receptor were positive in 46.0%. More than half was moderately differentiated tumor (59.2%) followed by poorly differentiated (39.4%). The tumor presented with T2 (60.5%) followed by T3 (25.0%) and T4 (7.8%).

Conclusions: Breast cancer among Lao women is characterized by large percentage of tiple negative and less susceptible to hormone receptors.

The empirical treatment with tamoxifen should be reconsidered since it would be less effective to patients.

More importantly, basic pathology services would be the first requirement in Lao PDR in order to provide adequate care.

248 Lymphoma Involving the Breast: An 18-Year Single-Institution Review Emphasizing Patients with Concurrent or Prior Carcinoma of the Breast

Christina Luffman[1], *Sean Raj*[2], *Vandana Dialani*[2], *Gabrielle Baker*[1]. [1]Beth Israel Deaconess Medical Center, Boston, MA, [2]Beth Israel Deaconess Medical Center

Background: Lymphoma involving the breast parenchyma (BL) is uncommon and most frequently affects post-menopausal women (F). Primary BL (PBL) is defined as BL limited to the breast (br), with (w/) or without regional lymph node (LN) involvement; systemic disease on staging is also included in PBL. Secondary BL (SBL) is defined as br involvement after a dx of lymphoma at a distant site (WHO 2012). Per the literature, the most frequent types of BL are diffuse large B-cell lymphoma not otherwise specified (DLBCL NOS), follicular lymphoma (FL), and extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (WHO 2012). Limited data is available regarding BL in patients (pts) w/ concurrent or prior diagnosis of br carcinoma (BrCa). The purpose of this study is to perform a single-institution review of BL, characterizing the subset of pts w/ concurrent or prior BrCa.

Design: Our pathology database was searched for diagnoses of BL (1998-2016). Demographic data and information regarding prior malignancy was noted.

Results: 35 pts w/ BL were identified: 97% were F, a broad age range was noted (35-90 years old [yo], median [med] 66yo). 54% were PBL (n=19; 35-90yo, med 63yo), and 46% were SBL (n=16; 40-81yo, med 63yo). The most common PBL were FL (26% of PBL; 14% of total [T]) and DLBCL (21% of PBL; 11% of T); the most common SBL were CLL/SLL and FL (each 38% of SBL; 17% of T). 20% (7/35) of pts also had a dx of BrCa: 4 had prior BrCa (57% of BrCa pts; 7-35yrs prior to BL dx; med 80yo) and 3 had concurrent BrCa (43% of BrCa pts; med 70yo). The 4 pts w/ prior BrCa had BL at the surgical site (n=3) or implant capsule (n=1). The type of BL at surgical site was CLL/SLL (n=2), FL (n=1), and implant-associated anaplastic large cell lymphoma (n=1). 1 of 3 concurrent cases had FL and invasive ductal carcinoma (IDC) in the br (IDC grade 2, ER+; LN negative for BrCa, positive for FL; 90yo). The remaining 2 concurrent cases had known CLL/SLL involving the br (1 w/ invasive lobular BrCa, grade 2, ER+, LN w/ BrCa and BL, 75yo; 1 BrCa w/ mixed features, grade 2, ER+, no LN evaluated, 76yo).

Conclusions: Our BL distribution is largely concordant w/ the literature. Whereas many of the case reports of BL in pts w/ BrCa note LN rather than br involvement, ours identifies pts w/ br involvement of both BL and BrCa. To the authors' knowledge, this represents the largest single-institution review of pts diagnosed w/ BrCa and BL.

249 Benign Breast Papilloma Diagnosed on Core Biopsy: Upgrade Rate and Risk Factors Associated with Malignancy on Surgical Excision

Christine E MacColl[1], *Phillip Williams*[2], *Amir Salehi*[3], *Parpia Parpia*[4]. [1]McMaster University, Hamilton, ON, [2]Juravinski Cancer Center, Hamilton, ON, [3]Montreal, QC, [4]McMaster University

Background: Evaluation of radiologically detected breast lesions is a common task in surgical pathology. Some lesions, which are benign on biopsy are associated with an upgrade to in situ or invasive malignancy on excision. An important lesion with potential for upgrade is the intraductal papilloma, which has published upgrade rates of

0% to 27%. Traditionally, benign papillomas have been managed with surgical excision in order to exclude malignancy with complete histologic examination. The wide range of published upgrade rates has raised uncertainty about the need for routine surgical management. This study aims to identify risk factors associated with upgrade and determine the upgrade rate of intraductal papillomas at our institution.

Design: The pathology reports of all breast core biopsies between 1-Jan-2006 and 31-May-2017 were searched electronically for a diagnosis of "papilloma" or "benign papillary lesion". Cases with associated malignancy or atypia were excluded. Clinical, radiologic and histologic details were recorded. For each patient who was managed surgically, the final pathology was retrieved and divided into two groups – benign or malignant. Available biopsy and resection slides from upgraded cases were reviewed by a blinded breast pathologist.

Results: 264 patients were diagnosed with a benign papilloma on breast core biopsy during the set time period. 180 patients were managed surgically. 21 patients had a diagnosis of in situ or invasive carcinoma on their final pathology, resulting in an upgrade rate of 12%. Logistic regression revealed that radiologically detected mass lesions with calcifications are at higher risk for upgrade than mass lesions without calcifications (odds ratio [OR] = 4.58, 95% confidence interval (CI) = 1.10, 19.10, p=0.037) and non-mass forming abnormalities (OR=1.75, 95% CI=0.48, 6.34, p=0.037). Additionally, advanced patient age was significantly associated with upgrade to malignancy (OR=1.07, 95% CI = 1.02, 1.18, p=0.003).

Conclusions: Risk factors associated with upgrade are radiologic identification of a mass lesion with calcifications and advanced patient age at diagnosis. Routine surgical excision of all papillomas is not recommended. Younger patients without high-risk features may benefit from clinical and radiologic follow-up alone. Accurate risk stratification will spare low-risk women unnecessary surgery and increase operating room availability for women with aggressive disease.

250 A Pilot Study Utilizing Addressing Mesenchymal CD90 (mCD90) Immunoexpression in Breast Carcinoma Tissue Microarray (TMA)

Nasma K Majeed[1], *OSAMA ELFITURI*[2], *Haoliang Xu*[3], *Alexandra Barabanova*, *Pradip Raychaudhuri*[4], *Grace Guzman*[5]. [1]University of Illinois at Chicago Hospital and Medical Center, Chicago, IL, [2]University of Illinois at Chicago, Chicago, IL, [3]University of Illinois-Chicago, Chicago, IL, [4]University of Illinois at Chicago, [5]University of Illinois, Chicago, IL

Background: Breast carcinoma is a leading cause of cancer-related mortality among women in the United States. Thy-1 (THYmocyte differentiation antigen 1) (CD90) is a T-cell marker that plays a role in cell-cell and cell-matrix interactions, inflammation, and fibrosis. Tissue microarray (TMA) is a reliable method for characterizing immunohistochemistry in research studies. Our aims were to assess CD90 in breast carcinoma and characterize its immunoexpression across tumor grades in TMA core samples.

Design: Tissue microarrays (TMAs) were designed and constructed from invasive breast carcinoma cases of which 76 cores survived processing. The tissue microarrays were stained with H&E and CD90 immunohistochemistry by standard methods and scanned with Hamamatsu scanner at 200X. The diagnosis of each core was verified using published criteria and semi-quantitative (visual) intensity of mCD90 was scored by independent observers using a scale of 0-3. Tumor characteristics data and mCD90 immunoexpression were merged and statistical analyses by Chi-square test was performed. A p-value of <0.05 was considered significant.

Results: mCD90 intensity scores were distributed as follows: 1+: 1/24 grades 1 and 2 tumors; 2+: 13/24 grades 1 and 2 and 1/52 grade 3 tumors; and 3+: 10/24 grades 1 and 2 and 51/52 grade 3 tumors (Pearson chi2 p<0.0001)(Table 1)

		Grade			
CD90	Score	Low (Grade 1,2)	High (Grade 3)	Total	
	0	0	0	0	
	1+	1	0	1	
	2+	13	1	14	
	3+	10	51	61	
	Total	24	52	76	
	Pearson	$chi^2(1)$	=	27.747	P-value<0.0001

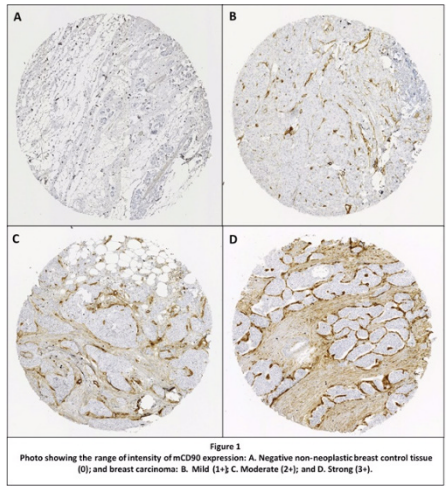


Figure 1
Photo showing the range of intensity of mCD90 expression: A. Negative non-neoplastic breast control tissue (0); and breast carcinoma: B. Mild (1+); C. Moderate (2+); and D. Strong (3+).

Conclusions: Increased expression of mCD90 was seen in breast carcinoma in comparison to non-neoplastic breast tissue. mCD90 was upregulated in high-grade (grade 3) versus low-grade (grade 1 and 2) tumors. These results provide support for further studies addressing the potential use of mCD90 as a biomarker for high-grade breast tumors.

251 TPX2 And Survivin Are Upregulated In Breast Cancers With Chromosomal Instability And Are Strong Predictors Of Poor Clinical Outcome And Unfavorable Molecular Status

Daniel Matson[1], *Katherine Pfister*[2], *Mark Burkard*[3], *Todd Stukenberg*[2], *Christopher Flynn*[1]. [1]University of Wisconsin Hospitals and Clinics, Madison, WI, [2]University of Virginia School of Medicine, [3]University of Wisconsin Hospitals and Clinics

Background: Chromosome instability (CIN) is a common form of genomic instability in which cells gain or lose whole or parts of chromosomes. CIN status is predictive of treatment outcome and prognosis in breast cancer, but there are no practical methods to measure CIN in patient tumors. Dr. Stukenberg and collaborators previously used a novel computational approach to search for genetic hallmarks of CIN in breast cancer. The results showed that the genes TPX2 and Survivin are upregulated in breast cancers with CIN. TPX2 and Survivin code for proteins with essential roles in mitotic spindle assembly and chromosome segregation during mitosis.

Design: We utilized 3 tissue microarrays (TMAs) collectively containing 372 breast cancer cases (in triplicate cores) containing the most common histologic subtypes. Cases are linked to 5 years of clinical follow-up data, treatment information, and tumor characteristics. Immunohistochemistry (IHC) was performed with anti-TPX2 (Sigma) and anti-Survivin (Abcam) antibodies. Cases with greater than 10% tumor cells positive for both TPX2 and Survivin were scored as high.

Results: High combined TPX2 and Survivin staining is significantly associated with increased tumor size, higher histologic grade, positive regional lymph nodes, more advanced stage, elevated Ki67 index, mutated p53, and negative ER and PR status. Clinically, high combined staining is significantly associated with premenopausal status at diagnosis, recurrence following initial treatment, reduced duration to recurrence in those who recur, and death due to breast cancer. In contrast, high combined TPX2 and Survivin status is not statistically associated with age at diagnosis, previous cancer diagnosis, family history of cancer, Her2 status, or duration from diagnosis to death in those who die of breast cancer.

Conclusions: Expression of TPX2 and Survivin has previously been shown to be elevated in breast cancers with CIN. We show that high combined staining for these proteins in patient breast cancers is strongly linked to poor clinical outcome and unfavorable molecular subtype. We believe the application of these two antibodies could provide a practical alternative to measuring CIN in the clinical setting.

252 CTLA-4+ Tumor Infiltrating Lymphocytes are Associated with Improved Prognosis in Triple Negative Breast Cancer

Patrick McIntire[1], *Timothy D'Alfonso*[2], *Zhengming Chen*[3], *Sandra J Shin*[4], *Paula Ginter*[5]. [1]Weill Cornell Medicine - New York Presbyterian Hospital, New York, NY, [2]Weill Cornell Medical College, New York, NY, [3]Weill Cornell Medicine, [4]Albany Medical College, Albany, NY, [5]Weill Cornell Medicine, New York, NY

Background: Triple negative breast cancer (TNBC) is associated with poorer prognosis and is more difficult to treat than hormone receptor positive and HER2 positive tumors. Studies have shown that a significant proportion of TNBC are associated with immune infiltrates and immune dysregulation is a key feature of breast cancer. Cytotoxic T lymphocyte antigen 4 (CTLA-4), an activated T-cell surface receptor, downregulates T-cell function and proliferation. Targeting and blockade of CTLA-4 with immunotherapy potentiates the anti-tumoral immune response. Although the efficacy of such therapies are currently under investigation, the role of CTLA-4+ TILs as a prognostic biomarker has yet to be firmly established, specifically in TNBC.

Design: Anti-CTLA-4 immunohistochemistry was performed on a representative whole tissue section from 73 cases of primary TNBC. Imaging and quantification of combined stromal and intratumoral CTLA-4+ TILs in 2.2 mm diameter circle hotspots (i.e., 10x HPF) were performed using HALO™ imaging analysis software (Indica Labs; Corrales, NM). TILs were enumerated as densities (absolute count/tissue area in micrometers^2). Statistical analyses were performed using disease free survival (DFS) and overall survival (OS) (range: 16 to 196 months, mean: 109 months, median: 118) as primary endpoints. Cox regression was used to estimate the hazard ratios (HRs) for the association between CTLA-4+ TIL counts and survival time. Kaplan-Meier curves were dichotomized into CTLA-4 high and CTLA-4 low cohorts based on the most significant split (log-rank test).

Results: Tumors high in CTLA-4+ TILs demonstrated statistically significant improved DFS (high vs low: HR = 0.26, 95% CI: 0.07 – 0.92, P = 0.0366) and OS (high vs low: HR = 0.33, 95% CI: 0.11 – 0.99, P = 0.0479). Kaplan-Meier curves also demonstrated a statistically significant survival benefit for DFS (*log-rank P* = 0.02) and OS (*log-rank P* = 0.04).

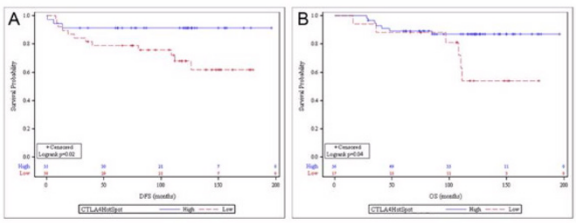


Figure 1. Kaplan-Meier Curves of Dichotomized CTLA-4+ TIL Counts for Disease-free survival (DFS) and Overall survival (OS) (A) CTLA-4+ hotspot Kaplan-Meier curve for DFS. (B) CTLA-4+ hotspot Kaplan-Meier curve for OS.

Conclusions: In this cohort, high CTLA-4+ TIL density was associated with favorable patient outcomes. Thus, CTLA-4+ TILs may serve as a potential prognostic biomarker in TNBC. Additional studies would be necessary to determine whether patients with TNBC high in CTLA-4+ TILs would benefit from targeted anti-CTLA-4 immunotherapy.

253 PD-1+ Tumor Infiltrating Lymphocytes and PD-1 mRNA Expression are Associated with Improved Outcomes in Triple Negative Breast Cancer

Patrick McIntire[1], *Timothy D'Alfonso*[2], *Zhengming Chen*[3], *Sandra J Shin*[4], *Paula Ginter*[5]. [1]Weill Cornell Medicine - New York Presbyterian Hospital, New York, NY, [2]Weill Cornell Medical College, New York, NY, [3]Weill Cornell Medicine, [4]Albany Medical College, Albany, NY, [5]Weill Cornell Medicine, New York, NY

Background: Immune checkpoint pathways including PD1 are an important mechanism by which cancer cells evade immune surveillance. In most solid tumors, PD-1 expression serves as an adverse prognostic marker. However, data in breast cancer are more conflicting, specifically in triple negative breast cancer (TNBC). We sought to evaluate the role of PD-1+ tumor infiltrating lymphocytes (TILs) and *PD-1* mRNA expression as prognostic biomarkers in TNBC.

Design: Anti-PD-1 immunohistochemistry was performed on a representative whole tissue section from 76 cases of primary TNBC. Imaging and quantification of combined stromal and intratumoral PD-1+ TILs from the tumor bed were performed using HALO™ imaging analysis software (Indica Labs; Corrales, NM). TILs were enumerated as densities (absolute count/tissue area in μm^2). Statistical analyses were performed using disease free survival (DFS) and overall survival (OS) as primary endpoints. Cox regression was used to estimate the hazard ratios (HRs) for the association between PD1+ TIL counts and outcome. Kaplan-Meier curves were separated into two clusters based on the top tertile level of PD-1+ TIL counts. *PD-1* gene expression data by U133 microarray for a cohort of 334 patients with TNBC were extracted from The Cancer Genome Atlas (TCGA) using cBioPortal (http://cbioportal.org). Clinical information for OS was extracted and Kaplan-Meier curves were generated by separating patients into two clusters based on the top tertile level of mRNA expression.

Results: PD-1+ TILs were significantly associated with improved DFS (HR=0.28, 95% CI: 0.07–0.69, P=0.02) and trended toward statistical significance for OS (HR=0.60, 95% CI: 0.24–1.14, P>0.05). When split by top tertile, PD-1+ TIL counts showed statistically significant improved DFS (high vs. low: HR=0.23, 95% CI: 0.05–0.99, P=0.048) and trended toward improved OS (high vs. low: HR=0.47, 95% CI: 0.13-1.70, P>0.05). Kaplan-Meier curves also demonstrated a statistically significant benefit for DFS (*log-rank P*=0.03) but not for OS (*log-rank P*>0.05). Kaplan-Meier curves generated from *PD-1* mRNA expression levels showed an association with OS (*log-rank P*=0.03). Clinical data on recurrence were not available to assess *PD-1* mRNA association with DFS.

Conclusions: Increased PD-1+ TILs and *PD-1* mRNA expression were associated with favorable patient outcomes in TNBC suggesting they may serve as prognostic biomarkers. The future of PD-1 as a predictive or companion biomarker in TNBC requires further study.

254 Excellent Inter-observer Concordance for CD8+ Tumor Infiltrating Lymphocyte Enumeration Using Digital Image Analysis on Hotspots in Triple Negative Breast Cancer

Patrick McIntire[1], *Elaine Zhong*[2], *Ami Patel*[3], *Francesca Khani*[4], *Timothy D'Alfonso*[5], *Zhengming Chen*[2], *Sandra J Shin*[6], *Paula Ginter*[4]. [1]Weill Cornell Medicine - New York Presbyterian Hospital, New York, NY, [2]Weill Cornell Medicine, [3]New York Presbyterian Hosp. Weill Cornell Medicine, New York, NY, [4]Weill Cornell Medicine, New York, NY, [5]Weill Cornell Medical College, New York, NY, [6]Albany Medical College, Albany, NY

Background: Tumor infiltrating lymphocytes (TILs) have emerged as prognostic in triple negative breast cancer (TNBC), with cytotoxic T-cell (CD8+) counts proving most prognostic among the TIL subtypes. The International TILs Working Group (ITWG) recommends enumerating stromal area occupied by TILs on H&E stained whole tissue sections. Although the ITWG guidelines are adequately prognostic, deficiencies in concordance among pathologists have been documented. Recent data suggest that CD8+ TIL hotspot counts are significantly associated with survival in TNBC. We assessed concordance of hotspot TIL counts among multiple pathologists. In addition, we evaluated the variation in TIL counts between multiple, distinct hotspots within a tumor section.

Design: Anti-CD8 immunohistochemistry was performed on a representative whole tissue section from 66 cases of primary TNBC. Imaging and quantification of combined stromal and intratumoral CD8+ TILs in 2.2 mm diameter circle hotspots (i.e., 10x HPF) were performed using HALO™ imaging analysis software (Indica Labs; Corrales, NM). TILs were enumerated as absolute counts and as densities (absolute count/tissue area in micrometers^2). For each case, six pathologists annotated a hotspot with the highest CD8+ immunoreactivity. In addition, a single pathologist annotated three separate hotspots within each tumor. Statistical analyses were performed using intraclass correlation coefficients (ICC).

Results: The ICCs calculated for density and absolute counts to determine concordance among pathologists' annotations were 0.96 and 0.97, respectively **(Figure 1).** In 32% of cases (21/66), all the hotspots annotated by the six pathologists were in nearly the same location **(Figure 2).** The ICCs calculated for density and absolute counts to determine variation in the three separate hotspots by a single observer were 0.95 each.

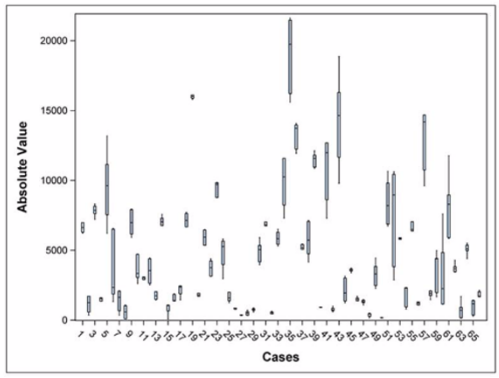


Figure 1. The distribution of CD8+ TIL absolute counts among six pathologists. The distribution of CD8+ TIL absolute counts enumerated from each of the six pathologists' annotation. Each boxplot represents 25th%, median and 75th% scores with the whiskers denoting the minimum and maximum scores of these six data points.

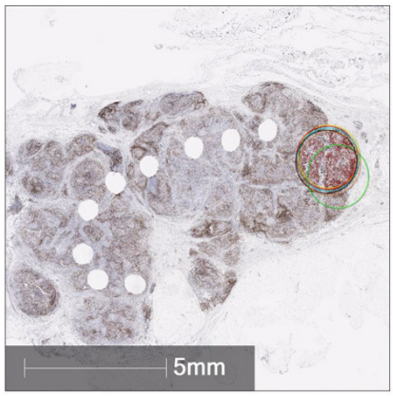


Figure 2. Example of Tumor Annotation in HALO™ on anti-CD8 stained whole tumor section. Low-power view of whole tumor section with 2.2 mm diameter circle hotspot annotations from 6 pathologists. CD8+ cells stain brown and cells enumerated by HALO™ are red.

Conclusions: Hotspot analysis showed excellent inter-observer concordance and pathologists commonly annotated nearly the same hotspot location. There was also very little variation among multiple hotspots within a single tumor section. Our findings suggest that evaluating a 2.2 mm diameter hotspot (corresponding to a 10x HPF) by digital image analysis is a reproducible method for quantification of CD8+ TILs. Additionally, within a single tumor section, multiple equivalent hotspots may be present yielding similar counts. This new methodology may represent an alternative to current ITWG guidelines for TIL enumeration in TNBC.

255 Metaplastic Breast Carcinomas Exhibit Activation of the HMGA2-IGF2BP2 Signaling Axis

Emily R McMullen[1], *Stephanie L Skala*[2], *Dafydd Thomas*[3], *Maria E Gonzalez*[4], *Celina G Kleer*[5]. [1]University of Michigan, Ann Arbor, MI, [2]University of Michigan, Ann Arbor, MI, [3]Univ. of Michigan Hosps, Ann Arbor, MI, [4]University of Michigan, [5]Michigan Medicine, Ann Arbor, MI

Background: Metaplastic breast carcinomas are triple negative cancers with poor outcomes, displaying metaplastic components including spindle, squamous, and/or chondroid. We demonstrated that MMTV-cre;Ccn6 knockout mice (CCN6 KO) develop mammary tumors recapitulating human spindle metaplastic carcinomas, with significant upregulation of the transcription factor HMGA2 (high mobility group AT-hook 2) and its pathway gene IGF2BP2 (insulin-like growth factor 2 mRNA binding protein 2) compared to wild-type mice. The HMGA2-IGF2BP2 axis promotes an epithelial to mesenchymal transition and migration in several malignancies. However, their expression and function in metaplastic carcinomas are unknown. We hypothesized that HMGA2 and IGF2BP2 proteins may be increased in metaplastic carcinomas, and may play a role in their development.

Design: We retrieved 31 metaplastic carcinomas resected from 1988-2015 at our institution. Tumors were reviewed and used to develop a tissue microarray (TMA), in duplicate. Immunohistochemistry for HMGA2 and IGF2BP2 was performed on TMA sections, CCN6 KO metaplastic carcinomas (n=6), and normal mammary glands (n=10). Expression was assessed on a four-point scale (0, 1, 2, 3), and staining ≥1 was considered positive. High-grade human breast cancer cells MDA-MB-231 were treated with recombinant CCN6 protein. Western blots were performed to detect HMGA2 and IGF2BP2 levels.

Results: HMGA2 was expressed in 15/31 (48%) and IGF2BP2 in 26/31 (84%) of human metaplastic carcinomas, independent of the predominant metaplastic component. HMGA2 and IGF2BP2 expression was negative in the normal epithelium adjacent to tumor (p=0.005). Of note, we found that both proteins were concordantly upregulated in 13/31 (42%) of human tumors. All CCN6 KO metaplastic carcinomas expressed HMGA2 and IGF2BP2. Similar to human tissues, no expression was detected in the normal epithelium. Western blots showed treatment of human breast cancer cells with CCN6 recombinant protein reduced the expression of HMGA2 and IGF2BP2 proteins, suggesting CCN6-mediated regulation of this signaling axis.

Conclusions: We identified a subset of human metaplastic carcinomas with concordant upregulation of the HMGA2-IGF2BP2 pathway, compared to negative normal epithelium. CCN6 KO mice recapitulate this subset of tumors, and constitute a good model to study the mechanism and to test novel treatment strategies in metaplastic carcinomas with activation of the HMGA2-IGF2BP2 axis.

256 Application of Morphologic Criteria for the Assessment of Menstrual Phase in Breast Tissue

Amanda L Meindl[1], *Jun Wang*[2], *Seema A Khan*[2], *Luis Blanco*[3]. [1]Northwestern University Feinberg School of Medicine, Chicago, IL, [2]Northwestern University Feinberg School of Medicine, [3]Northwestern University Feinberg School of Medicine, Chicago, IL

Background: Hormonally responsive biomarkers of breast cancer risk (e.g. Ki-67) change with menstrual cycle in premenopausal women. Biomarker studies would be facilitated if menstrual cycle phase is considered in the analysis, but this is usually not available for archival samples. We applied morphologic criteria by Ramakrishnan et.al. (*Mod Pathol* 2002;15:1348–1356) to four quadrants in the contralateral unaffected breast (CUB) tissue of women diagnosed with breast cancer or representative sections from reduction mammoplasty (RM) specimens. Menstrual phase by morphology (MPM) was compared to menstrual phase by hormone levels (MPH) determined at the time of surgery. In addition, we compared MPM among the four quadrants.

Design: 79 patients (mean age=43.3, range=19-56) were evaluated. Breast lobule morphology was scored in four sections of the CUB or RM specimens. Criteria included epithelial-myoepithelial distinction, myoepithelial vacuolation in the acini and lobules, stromal edema, stromal inflammatory infiltrate, apoptosis and mitosis (Table 1). Total morphology score corresponded to a menstrual phase (Table 2). The correlation between MPM and MPH was tested using Spearman's rank correlation test with statistical significance at $p<0.05$.

Results: The overall consistency between MPM in the UOQ and MPH is presented in Table 3; the correlation between MPM in the UOQ and MPH is significant (R=0.409, p=0.0004). Similarly, the overall consistency between MPM average score of the four quadrants and MPH is 48.1% (38/79), with 52.9% (9/17) in EF phase, 56.8% (25/44) in LF phase, and 22.2% (4/18) in luteal phase (LP); the correlation is significant (R=0.371, p=0.0014). The MPM scores were consistent among the four quadrants in 66% (52/79) of cases, with highest consistency in LP at 88% (7/8), 70% (32/46) for LF phase, and lowest in EF phase at 52% (13/25).

Table 1. Morphologic Criteria for the Assessment of Menstrual Phase in Breast Tissue

Morphologic Criteria	0	1	2	3
Epithelial -myoepithelial distinction	2 distinct layers not seen	2 layers seen in <30% of the lobule	2 layers seen in 31–74% of the lobule	2 layers seen in >75% of the lobule
Myoepithelial vacuolation in the acini	absent	vacuolation in <30% of the acinus	vacuolation in 31–74% of the acinus	vacuolation in >75% of the cells
Myoepithelial vacuolation in the lobules	absent	vacuolation in <30% of the lobules	vacuolation in 31–74% of the section	vacuolation in >75% of the section
Stromal edema	absent	mild	moderate	marked
Stromal infiltrate	absent	mild	moderate	marked
Apoptosis	absent	occasional, 1 or 2	frequent, >3	N/A
Mitosis	absent	occasional, 1 or 2	frequent, >3	N/A

Table 2. Distribution of the Individual Phases by Morphologic Score and by Days

Menstrual Phase	Total Score by Morphology	Days In Menstrual Cycle
Early Follicular	0-5	0-5
Late Follicular	6-9	6-15
Early Luteal	10-15	16-24
Late luteal	16-19	25-28

Table 3. Consistency Between Menstrual Phase by Morphology in the Upper Outer Quadrant (UOQ) and Menstrual Phase by Hormones

Phase by Hormones	Phase by Morphology Score in the UOQ			Total	Consistency
	Early Follicular	Late Follicular	Luteal		
Early Follicular	8	9	0	17	47.1%
Late Follicular	11	28	5	44	63.6%
Luteal	2	12	4	18	22.2%
Total	21	49	9	79	50.6%

Conclusions: Given the consistency in MPM among the four quadrants, assessment of MPM may be applied to any representative section of the breast specimen. Further, given the significant correlation of the average MPM among the four quadrants and MPH and between MPM in the UOQ and MPH, assessment of MPM can better facilitate biomarker studies of breast cancer risk in archival tissue. These results

provide encouraging validation of the use of morphologic criteria for menstrual phase assignment in breast tissue. This could be improved upon with quantitation of gene expression during different phases of the menstrual cycle, and this work is currently under way.

257 Histopathologic Characterization of Breast Cancers in Patients with Non-BRCA Germline Mutations

Alice Meiss[1], *Martha H Thomas*[2], *Lydia Higgs*[3], *Kristen Atkins*[4], *Anne Mills*[4]. [1]UVA, [2]UVA, Charlottesville, VA, [3]University of Virginia, [4]Charlottesville, VA

Background: As genetic testing for hereditary cancer syndromes becomes more commonplace, germline mutations in genes other than *BRCA1* and *BRCA2* are increasingly identified in breast cancer patients. While histopathologic features of *BRCA*-mutated breast cancers have been well-characterized, much less is known about non-*BRCA*-related hereditary breast cancers. We herein investigate the histopathologic characteristics of primary breast cancers in women with non-*BRCA* germline mutations.

Design: The institutional genetic counseling database was retrospectively reviewed to identify breast cancer patients found to have a non-*BRCA* germline mutation implicated in hereditary predisposition to breast cancer. This database included all women who had elected to undergo germline testing from 2015-2017. Cases with available slides were reviewed and assessed for histologic subtype, grade, lymphocytic infiltration, stromal reaction, and background in situ carcinoma.

Results: 612 women with a personal history of breast cancer underwent germline testing from 2015-2017. 17 women (2.8%) with 20 cancers were found to have mutations in genes other than *BRCA1/2*. These mutations represent a spectrum from clinically actionable mutations to mutations of unknown significance. Average age at diagnosis was 51 (range: 27-77). 9 genes were involved: *ATM, CHEK2, PALB2, TP53, BLM*, *BMPR1A, BRIP1, MUTYH*, and *RAD50*. (TABLE) The majority (79%) of tumors were grade 1-2. 35% were either lobular or ductal with lobular features. Stromal responses varied from absent to desmoplastic to sclerotic. 69% of cases had an associated in situ component. With the exception of a brisk lymphocytic response in *BLM* and *TP53*-mutated cancers, the tumors showed either absent or only mild lymphocytic infiltrate.

FOR TABLE DATA, SEE PAGE 121, FIG. 257

Conclusions: The majority of non-*BRCA*-related hereditary breast cancers are low to intermediate-grade and represent the patient's sentinel malignancy. Lobular features are seen in a significant subset. In contrast to BRCA-associated cancers, high-grade, immunogenic carcinomas are uncommon in the context of other hereditary malignancies except in the setting of *BLM* and *TP53* mutations. Larger, multi-institutional studies are needed to further characterize the clinicopathologic features of non-*BRCA*-related hereditary breast cancers and investigate whether histomorphology can inform testing algorithms; data from this study suggests that many of these tumors have a banal appearance that does not distinguish them from sporadic malignancies.

258 Frequency and Significance of Basal Phenotype in ER-positive Breast Cancer

Anne-Sophie Meyer[1], *Mark Kriegsmann*[1], *Nina Waldburger*[2], *Audrey Jung*[3], *Sabine Behrens*[4], *Jenny Chang-Claude*[3], *Hans-Peter Sinn*[5]. [1]University Hospital Heidelberg, [2]University of Greifswald, [3]German Cancer Research Center, [4]German Cancer Research Center, Heidelberg, Baden-Württemberg, [5]University Hospital Heidelberg, Heidelberg, Baden-Württemberg

Background: While basal phenotype is frequent in triple-negative breast cancers, it is much more infrequently observed in breast cancers with hormone receptor-positive status. Data in the literature regarding the frequency of this situation are scarce, and its clinical significance is unknown. Therefore, we have analyzed a large cohort of primary breast cancers for the presence of basal phenotype in ER+ breast cancers.

Design: Formalin fixed and paraffin embedded tumor tissue was collected from the population-based mammary carcinoma risk factor investigation (MARIE) study. A total of 1428 invasive breast cancers were analyzed. Cores with insufficient tumor cells or only in situ-carcinoma were excluded. 1047 cases were evaluable for IHC subtyping (using ER, PR, HER2, Ki-67) and additional markers (Ck5/6, Ck14, EGFR, p53). Using a set of tissue microarrays (TMAs) containing duplicates of 1 mm cores, tumors were classified as hormone receptor positive when at least 10% of tumor cells were ER-positive. Based on ASCO recommendations, HER2 positivity was assumed for tumors with IHC score of 3+ or amplification of HER2 chromogenic in situ-hybridization with a ratio of $\geq$ 2.0 in a dual color assay. Basal phenotype was considered present when at least 10% of the tumor area was positive with basal cytokeratins, either Ck5/6 or Ck14.

Results: Basal phenotype was observed in 8,9% (49/449) of breast cancer with ER+/HER2- status, and less frequently with tumors of Luminal B-phenotype. In HER2+ breast cancer, basal phenotype was present in 11,4% (17/149, and more frequent in HER2+/ER- cancers than in HER2+/ER+ cancers (13,9% vs. 9,1%). Basal phenotype was significantly associated with accelerated tumor proliferation (Ki-67 > 20%), and was present in 17.1% of tumors with Ki-67 > 20% but only in 11.8% of tumors with lower Ki-67 proliferation rates. In Luminal-Type breast cancer, ER+/HER- basal phenotype was not significantly associated with accelerated tumor proliferation (Ki-67 > 20%), being present in 9.0% of tumors with Ki-67 > 20%, and 8.5% of tumors with lower Ki-67 proliferation rates. No significant differences in 5-year overall survival was observed when comparing ER+/HER- basal-like and non-basal like breast cancers (85.4% vs. 88.3%, p=0.5) cancer.

Conclusions: About 9% of ER+ breast cancers show a basal phenotype, irrespective of HER2 status. This is highly correlated with tumor proliferation but not in ER+ breast cancers, and has no significant impact on survival after 5 years in ER+ breast cancers.

259 Stability of HER2 Expression in Residual Breast Cancer Following Neoadjuvant Anti-HER2 Therapy.

Mariam o Mir[1], *Sunati Sahoo*[2], *Yisheng Fang*[3], *Yan Peng*[2], *Helena Hwang*[4], *Agata Czapla*[4], *Prasad Koduru*[3], *Venetia Sarode*[5]. [1]UT Southwestern, Dallas, TX, [2]UT Southwestern Medical Center, Dallas, TX, [3]UT Southwestern Medical Center, [4]UT Southwestern, Dallas TX, [5]University of Texas Southwestern, Dallas, TX

Background: There are conflicting reports on the stability of HER2 expression after neoadjuvant anti-Her2 therapy (NAHT) in patients with HER2 positive breast cancer. The assessment of HER2 after NAHT may have treatment implications. Loss of HER2 expression has been associated with higher risk of relapse. The aim of this study is to determine HER2 expression in residual tumors of patients treated with NAHT. We also evaluated HER2 status in residual tumors of patients treated with conventional neoadjuvant chemotherapy (NCT) only.

Design: A retrospective analysis of data on HER2 positive invasive breast cancer from 2011-2016 was performed. Prognostic markers (ER, PR and HER2) were evaluated by IHC using standard protocols on pretreatment biopsies and residual tumors after surgery. Positive hormone receptor (HR) and HER2 overexpression was defined as per the CAP/ASCO guidelines. HER2 3+ and 2+ IHC results were confirmed by FISH analysis.

Results: We identified 292 HER2+ breast cancer cases, 167 (57.1%) received neoadjuvant therapy; 135 (81%) with NAHT and 32 (19%) with NCT only. Pathologic complete response (pCR) was achieved in 59 (35%), of which 56 (95%) were in the NAHT and 3 (5%) in the NCT group (p=0.0004). The rate of pCR was higher in the HR-/HER2+ group, 54% (32/59) vs 46% (27/59) in the HR+/HER2+ group (p=0.461). In the NAHT group, 56 of 79 (71%) patients with residual tumor were re-tested for HER2: 41 (73%) remained HER2 positive and 15 (27%) became HER2 negative. In the NCT group, 15 of 29 (52%) residual tumors were retested for HER2: 14 (93%) remained positive and 1 (7%) was negative (p=0.163).

Conclusions: A high rate of pCR was achieved in the NAHT group. HER2 overexpression remains unchanged in the majority of the patients with residual tumor. The stability of HER2 supports the current practice of extended anti-Her 2 therapy after surgery. Heterogeneity in HER2 expression may be the reason for loss of HER2 in those treated with NAHT. The emergence of HER2 negative clones might be an indication for adjuvant chemotherapy in addition to continuing anti-HER2 therapy.

260 Digital Support for the Diagnosis of Ductal Carcinoma In-Situ (DCIS) and Tumor Grading for Improved Patient Care

Shachi Mittal[1], *Andre Kajdacsy Balla*[2], *Rohit Bhargava*[3] [1]Champaign, IL, [2]Univ. of Illinois, Chicago, IL, [3]UIUC

Background: The limited use of digital microscopy in understanding and diagnosing cancer arises from the lack of technology to capture multiple tissue compartments and detailed morphological and biochemical signatures. Currently, it is also difficult to differentiate between *in-situ* and invasive tumors. Even if the lesion is classified as *in-situ*, it is not possible to predict if all the DCIS cells will progress towards an invasive phenotype. Therefore, precise diagnosis of *in-situ* lesions and predictive models for their progression is indispensable for early detection and subsequently improved patient outcome. Infrared (IR) spectroscopic imaging, in particular, combines the morphologic imaging capability of optical microscopy and the chemical specificity of vibrational spectroscopy to provide quantitative chemical tissue characterization. Here we present, a digital diagnostic toolbox for precise DCIS detection and tumor grading.

Design: Materials: Breast tissue microarrays were imaged using high definition infrared imaging. Consecutive histology sections were stained for H&E and immunohistochemical stains.

Data Analytics: IR images were manually annotated under the supervision of a pathologist for building training models. Specific features, i.e. linear transformations of various IR bands were used for supervised learning to digitally differentiate between DCIS and invasive tumors, and to grade the invasive tumors.

Results: Figure 1 illustrates the spatial performance of the developed diagnostic model for differentiating between DCIS cases and invasive tumors. The top row represents classified images of four patient cases out of the 100 used for model development, two DCIS and two invasive cases. A five class model separating benign, DCIS, invasive tumor, stroma and "others" (other cellular compartments like necrosis, RBCs, etc.) is utilized. Table 1 represents the confusion matrix on all the 100 cases with the diagonal elements indicating true positives. Similarly, figure 2 and table 2 depicts the performance of tumor grading model wherein grade 2 and grade 3 cases are digitally identified by using infrared spectroscopic imaging data coupled to machine learning.

Table1. Confusion matrix of DCIS detection model

Classes	Benign	DCIS	Invasive Tumor	Stroma	Others
Benign	97.5	0.0	1.5	0.5	0.5
DCIS	0.1	96.8	2.7	0.2	0.2
Invasive Tumor	3.3	5.1	88.0	1.6	1.9
Stroma	0.9	0.3	3.7	92.6	2.5
Others	0.3	0.0	0.4	1.4	98.0

Table2. Confusion matrix of tumor grading model

Classes	Benign	Grade2	Grade3	Stroma	Others
Benign	98.2	0.9	0.2	0.3	0.4
Grade2	2.4	85.5	10.0	0.8	1.3
Grade3	0.1	6.8	88.7	2.2	1.3
Stroma	0.8	1.7	2.6	92.7	2.2
Others	0.1	0.1	0.3	1.4	98.0

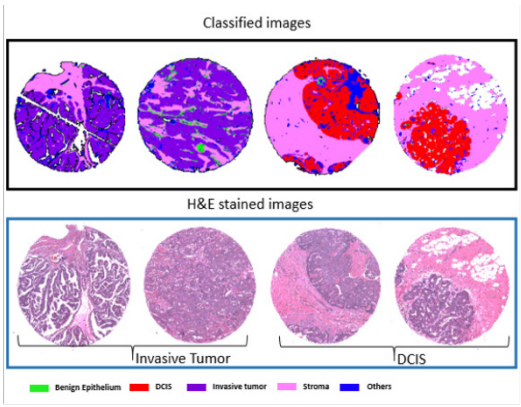


Figure1. DCIS detection model with classified images (top row) and corresponding H&E stained images (bottom row)

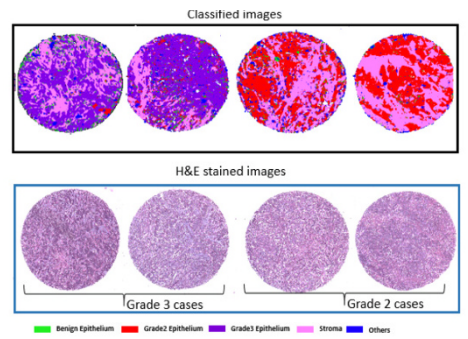


Figure 2. Tumor grading model with classified images (top row) and corresponding H&E stained images (bottom row)

Conclusions: The presented study will open new avenues for rapid, objective and automated diagnostic support and prognostic information to improve patient care. This will address the long recurring need of reducing inter and intra- observer variability, thereby impacting surgical treatment options and hence patient outcomes.

261 Clinicopathologic Features, Management and Outcomes of Breast Secretory Lesions with and without Atypia

Kelly Mooney[1], *Chieh-Yu Lin*[2], *Richard Sibley*[2], *Mara Rendi*[3], *Kimberly Allison*[1]. [1]Stanford University, Stanford, CA, [2]Stanford University Med. Ctr., Stanford, CA, [3]University of Washington

Background: Benign secretory lesions of the breast include pseudolactational change (PLC) and cystic hypersecretory change (CHC). These lesions can occur together and can exhibit cytologic atypia. While cystic hypersecretory carcinoma *in situ* (CHC*is*) has been associated with these secretory lesions, their clinical and pathologic characteristics are incompletely characterized.

Design: A retrospective review of PLC, CHC, and hybrid PLC/CHC with and without atypia diagnosed on breast biopsy at two academic institutions over 14 years was performed, excluding cases in the setting of pregnancy and lactation. Slides were reviewed, and clinical, histologic, and outcomes data were recorded. Statistical analyses were performed using two-tailed t-tests.

Results: Eighteen non-atypical and 15 atypical secretory lesion cases were identified (see table 1). The mean age at diagnosis was 46.5 years. Most biopsies targeted calcification (60%), or a mass (30%). Lesions were considered the imaging target (vs incidental) in 9/15 (60%) of atypical lesions and 8/18 (44%) non-atypical lesions. Non-atypical secretory lesions were predominantly isolated PLC (61%), or PLC associated with apocrine metaplasia, columnar cell change, or sclerosing adenosis (28%). None of these were hybrid PLC/CHC lesions. In contrast, many atypical cases were hybrid (8/15, 53%). Atypical lesions were more likely to undergo excision (69% vs 7%, p=0.001). The single excised non-atypical lesion was isolated PLC in the setting of a palpable mass, which demonstrated focal CHC on excision. Of 9 excised atypical lesions, 2 were upgraded to CHC*is* – one was an atypical hybrid lesion in the setting of a 0.9 cm mass, and one was CHC with associated atypical ductal hyperplasia in the setting of a palpable mass. No atypia or carcinoma *in situ* was seen on excision of isolated atypical PLC (n=5). All patients were alive with BI-RADS score 1-2 reported at follow-up examination (average 48.5 months).

Table 1. Clinicopathologic Features and Outcomes of Breast Secretory Lesions with and without Atypia Diagnosed on Biopsy

Secretory Lesions	Non-atypical (n = 18)	Atypical (n = 15)
Diagnoses	12 PLC 5 PLC + other benign 1 CHC	9 Atypical hybrid PLC/CHC 5 Atypical isolated PLC 1 Atypical CHC + ADH
Mean imaging span [range]	Calcification: 13.8 mm [4-50] Mass: 5.5 mm [5-6]	Calcification: 4.8 mm [3-10] Mass: 10 mm [9-11]
Lesional calcification on histology	13/18 (72%)	5/15 (33%)
Secretory lesion = imaging target	8/18 (44%)	9/15 (60%)
Excision diagnoses	1 Focal CHC	5 Benign 2 PLC 2 CHCis
Abbreviations: ADH, atypical ductal hyperplasia; CHC, cystic hypersecretory change; PLC, pseudolactational change.		

Conclusions: PLC, CHC and hybrid PLC/CHC occur as both incidental and targeted findings diagnosed in the setting of breast biopsy for calcifications and masses. CHC and hybrid PLC/CHC with cytologic atypia seen in the setting of biopsies for a mass were associated with cystic hypersecretory carcinoma *in situ* on excision in 2/6 (33.3%) cases. Non-atypical secretory lesions identified in biopsies for calcifications had stable imaging or negative excisions on follow-up.

262 Adenomyoepithelial Tumors of the Breast Harbor ARID1A Mutations and PI3K/AKT Pathway Alterations

Juan Miguel Mosquera[1], *Samaneh Motanagh*[2], *Olivier Elemento*[3], *Sandra J Shin*[4], *Patrick McIntire*[5]. [1]Weill Cornell Medical College, New York, NY, [2]Weill Cornell Medicine, New York, NY, New York, NY, [3]New York, NY, [4]Albany Medical College, Albany, NY, [5]Weill Cornell Medicine - New York Presbyterian Hospital, New York, NY

Background: Adenomyoepithelial (AME) tumors of the breast are uncommon and span the morphologic spectrum of benign, atypical, pre-invasive, and invasive forms. In exceptionally rare cases, these tumors metastasize to regional lymph nodes or distant sites. In the era of genomic characterization, little is known about AME tumors and in particular, their metastatic counterparts. The aim of this study was to provide insight into the molecular underpinnings of AME tumors and their neoplastic evolution into metastatic disease.

Design: Five cases of AME tumors of the breast (benign-1, atypical-1, *in situ*-1, invasive-1, invasive with paired axillary lymph node metastasis-1) were identified in our files. The six samples were interrogated using the Oncomine Comprehensive Assay v3 (ThermoFisher).

Results: Two separate malignant cases (invasive-1 and metastasis-1) showed the same loss-of-function *ARID1A* indel. The atypical and AME *in situ* cases harbored the same gain-of-function *PIK3CA* mutation. The malignant AME *in situ* also showed EGFR amplification. Both benign and invasive AME (case without metastasis) shared the same gain-of-function *AKT1* variant. Moreover, the same gain-of-function *HRAS* mutation was present in the atypical AME and invasive AME with metastasis. Interestingly, both samples of invasive AME and corresponding metastasis shared loss-of-function *NOTCH3* indel. No fusion drivers were detected. Average read depth was 1082x.

Conclusions: We describe the molecular characteristics of the spectrum of AME tumors of the breast, which harbor alterations in *ARID1A* and PI3K/AKT pathway. Our findings are clinically relevant given the current options of targeted therapy in rare instances where malignant AME tumors of the breast progress.

263 Race and Body Mass Index Have No Significant Independent Association with Distant Metastasis in Triple Negative or Non-Triple Negative Breast Cancer

Nitai Mukhopadhyay[1], *Jaime Singh*[2], *Michael Idowu*[3]. [1]VCU Health Systems, [2]Virginia Commonwealth University Health Systems, Richmond, VA, [3]Virginia Commonwealth Univ Health System, Richmond, VA

Background: Obesity has been associated with increased risk of developing breast cancer, especially in post-menopausal women, and has been linked to breast cancer specific mortality (BCSM). Obesity may contribute to increased levels of estrogen due to over-expression of pro-inflammatory cytokines, insulin resistance, excessive aromatization of adipose tissue and oxidative stress. Weight control is encouraged as part of overall management of breast cancer patients. There is racial disparities in BCSM with African American women having the highest BCSM rate compared to other races. The aim of this study was to evaluate the correlation between race and Body Mass Index (BMI) with distant metastasis in triple negative and non-triple negative breast cancer.

Design: Breast cancer cases with adequate clinical-pathologic follow-up information were retrieved between 2005 and 2013 (age range at diagnosis: 25 to 92 years). Follow up information including race (Black vs White), BMI (range: 14.2 to 65.9 kg/m^2), and occurrence of distant metastasis was collected via patient electronic medical records and the Cancer Registry. Logistic regression was used to calculate statistical significance. A p-value <0.05 was considered significant.

Results: A total of 185 cases were reviewed. Race and BMI did not have a significant association with distant metastasis in triple negative or non-triple negative breast cancer (Tables 1a-1c).

Table 1a. Logistic regression of correlation between race and BMI with breast cancer distant metastasis for triple and non-triple negative phenotypes

N=185	Analysis of maximum likelihood estimates	Odds ratio (95% confidence limits)	Correlation to Distant metastasis (p value)
Race (Black vs White)	-0.2433	0.615 (0.334-1.130)	0.1173
BMI	0.00977	1.010 (0.970-1.051)	0.6336

Table 1b. Logistic regression of correlation between race and BMI with breast cancer distant metastasis for non-triple negative phenotypes

N=138	Analysis of maximum likelihood estimates	Odds ratio (95% confidence limits)	Correlation to Distant metastasis (p value)
Race	-0.1815	0.696 (0.321-1.506)	0.3573
BMI	0.0249	1.025 (0.967-1.087)	0.4007

Table 1c. Logistic regression of correlation between race and BMI with breast cancer distant metastasis for triple negative phenotypes

N=47	Analysis of maximum likelihood estimates	Odds ratio (95% confidence limits)	Correlation to Distant metastasis (p value)
Race	-0.1358	0.762 (0.212-2.746)	0.6779
BMI	-0.0210	0.979 (0.918-1.045)	0.5268

Conclusions: Race and BMI were not observed to be useful independent predictors of distant metastasis in triple negative and non-triple negative breast cancer. There may be other confounding factors associated with reduced overall survival.

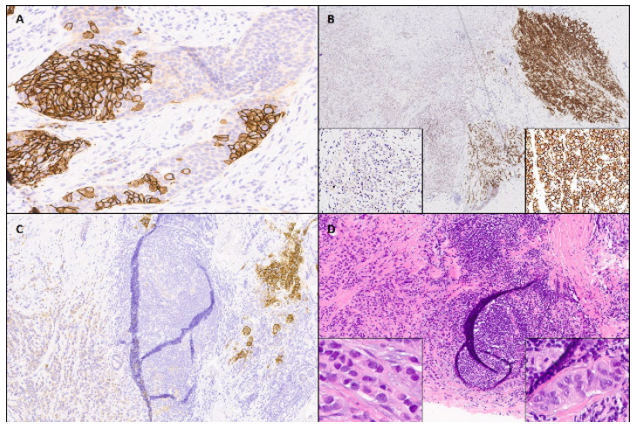
264 HER2 Intratumoral Heterogeneity in Breast Cancer: Clinicopathologic Characteristics

Kristen E Muller[1], *Jonathan Marotti*[2], *Laura Tafe*[3]. [1]Dartmouth-Hitchcock Medical Center, Lebanon, NH, [2]Dartmouth-Hitchcock Medical Center, Lebanon, NH, [3]Dartmouth-Hitchcock Med.Ctr., Lebanon, NH

Background: HER2 intratumoral heterogeneity (ITH) in breast cancer is a rare occurrence; the clincopathologic characteristics, management, and clinical significance of tumors with HER2 ITH are not well-defined. The aim of this study was to review the features of breast cancers with HER2 ITH at our institution.

Design: Our pathology database was searched to identify breast cancers with HER2 ITH. Cases had to demonstrate HER2 ITH by both upfront *HER2/neu* FISH and reflex immunohistochemistry (IHC). Clinical and pathologic information was extracted from electronic records. Slides were reviewed to document percentage of overall HER2 positivity, spatial patterns and corresponding morphologic heterogeneity.

Results: Ten cases with HER2 ITH were identified during a 6.75 year period. The tumors consisted of invasive ductal carcinomas (IDC, 8/10) and invasive lobular carcinomas (ILC, 2/10), and were classified as high (7/10), intermediate (2/10) or low grade (1/10). Nine cases were estrogen and progesterone receptor positive, while one was negative. *HER2/neu* FISH was performed in all cases with a mean *HER2/CEP17* ratio of 5.6 ± 3 (range 2.5-11.5) in amplified populations. Reflex HER2 IHC confirmed subclones with HER2 overexpression ranging from <5% to 50% of tumor cells in predominantly clustered groups (Figure 1A,B). Three tumors displayed morphologic heterogeneity corresponding to HER2 heterogeneous populations. One case of conventional ILC contained HER2-positive clones in cells with pleomorphic features and tumor within lymphatics (Figure 1C,D). The second case showed HER2 positivity in micropapillary areas in a tumor with mixed ductal, papillary and micropapillary morphology. The third case showed HER2 positivity in a more solid and higher grade area within an otherwise predominantly low grade IDC. Seven of ten patients received anti-HER2 therapy, two of which received neoadjuvant anti-HER2 therapy; both of these patients had a partial pathologic response. Clinical follow-up (mean 33.4 ± 20.2 mos, range 8-70 mos) revealed eight patients with no evidence of disease and two with metastatic disease (HER2-positive brain metastasis and HER2-negative supraclavicular metastasis).



Conclusions: HER2 ITH occurred predominantly in high grade IDC and was seen in tumors with morphologic heterogeneity. The possibility of HER2 ITH should be considered in such cases, however, the clinical significance of identifying HER2 ITH remains unclear.

265 Expression of miRNA-150-5p in triple negative breast cancer of African-American and Non-Hispanic White patients

Tammey Naab[1], *Yasmine M Kanaan*[2], *Luciane Cavalli*[3], *Muhammad M Hassan*[4]. [1]Mc Lean, VA, [2]Howard University Hospital, [3]Georgetown University, [4]Howard University Hospital, Washington, DC

Background: Triple Negative Breast Cancer (TNBC), a clinically aggressive subtype of breast cancer, disproportionately afflicts African American (AA) women compared to Non-Hispanic Whites (NHW). MiRNA-150-5p has been shown to control the expression of driver cancer genes involved in critical signaling pathways, including the ones associated with metastasis development.

Design: In this study we determined by qRT-PCR the expression status of miRNA-150-5p in 47 cases of TNBC, 32 from AA and 15 from NHW patients, obtained from the pathology bank of Howard University and Lombardi Comprehensive Cancer Center, Washington DC.

Results: MiR-150-5p expression alterations were observed in 59.4% and 80% of the TNBC of AA patients and NHW patients, respectively; in both groups of patients down-regulation was more frequently than up-regulation, with no significant difference between the groups (P>0.05). In both groups no association was observed among miRNA-150-5p expression and age, tumor size and Elston grade, however a significant association was observed between down-regulation of miRNA-150-5p and the presence of lymph node metastasis in the AA group (P=0.03).

Conclusions: Our findings indicate that miR-150-5p is frequently down-regulated in TNBC, with no significant differences in expression in AA and NHW women. Further studies are required to support its role as a tumor suppressor gene in these tumors in association with metastasis development.

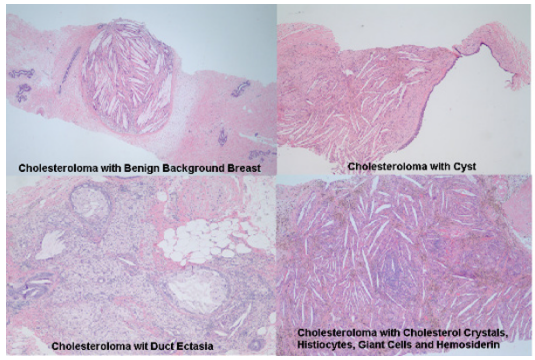
266 Mass Forming Cholesteroloma of the Breast: A Case Series of 43 Patients with Radiology Correlation

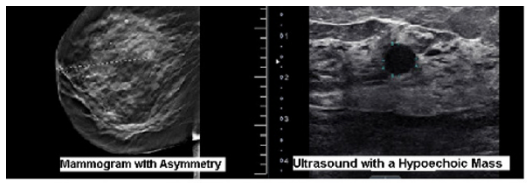
Gahie Nam[1], *Tisha M Singer*[2], *Ana Lourenco*[2], *Yihong Wang*[3]. [1]Alpert Medical School of Brown University, Providence, RI, [2]Rhode Island Hospital, [3]Rhode Island Hospital, Providence, RI

Background: Cholesteroloma is an uncommon benign breast lesion or part of an inflammatory/reactive process with unclear etiology. The purpose of this study was to review cholesteroloma of the breast with clinical and radiologic correlation.

Design: Databases were searched for "cholesterol cleft" from 2010 to 2017. 80 cases were retrieved from a total of 9850 breast core needle biopsies (CNB). The slides were reviewed and 46 cases from 43 patients with cholesterolomas as primary mass-forming lesions were selected. Inflammatory lesions of breast (e.g. granulomatous mastitis), minor cholesterol clefts formation and non-mass forming stereotactic biopsy for calcifications were excluded.

Results: The mean patient age was 57.4 (36-90) with female to male ratio of 42:1. The mean body mass index was 25.5 kg/m^2 (17.7-41.5). The mean blood cholesterol level was 200 mg/dl (152-291). All cases were ultrasound guided CNB for mass forming lesions by radiology. The mean mass size was 9 mm (3-23). Cholesteroloma as solitary mass was in 19 cases (41%), followed by 8 cases (17%) with associated ruptured macrocyst, 7 cases (16%) with dilated ducts/macrocyst without evidence of rupture, 3 cases (7%) with duct ectasia, 2 cases (4%) with fat necrosis and 3 cases (7%) with radial scar/complex sclerosing lesion. Fibrocystic changes were a common finding in the background (20%). Radiologically, 35 cases (76%) presented as solid mass lesions and 10 cases (24%) as cystic lesions. Five of the cystic lesions were found to be associated with macrocyst. The diagnostic mammogram or ultrasound BI-RADS assessment was most frequently 4, suspicious (82%, n=38), followed by 5, highly suggestive of malignancy (11%, n=5). The corresponding histology of the subset of BI-RAD 5 cases were: 3 solitary lesions and 2 cholesteroloma with associated ruptured macrocysts. Of the 16 patients with imaging follow-up, 94% had negative or benign findings with one patient having a persistent mass. One patient underwent excision because of a concurrent radial scar and that showed cholesteroloma associated with macrocysts.





Conclusions: Cholesterolomas of the breast are rare (prevalence= 0.47%) and can be seen on CNBs. The imaging can be of solid or cystic lesions and sometimes mimics cancer. Macrocyst/dilated duct and duct ectasia are the most common associated changes, which could explain the pathogenesis. The recognition of this lesion and radio-pathological correlation can help us better understand this entity and distinguish it from its mimickers.

267 Breast Cancer Risk Associated with Benign Papillomas Initially Diagnosed on Core Needle Biopsy

Leyla Nasehi[1], *Nidhi Sharma*[1], *Paulette L Lebda*[1], *Benjamin C Calhoun*[2]. [1]Cleveland Clinic, [2]University of North Carolina, Chapel Hill, NC

Background: The low upgrade rates in recent studies of benign intraductal papilloma (IDP) diagnosed on core needle biopsy (CNB) suggest immediate surgical excision may not be mandatory. The long-term risk for patients with benign IDP who are not upgraded on excision is not well defined. The goals of this study were: 1) to report the rate of upgrade to carcinoma [ductal carcinoma in situ (DCIS) and invasive carcinoma (IC)] in excisions of benign IDP diagnosed on CNB, and 2) to report the cumulative breast cancer (BC) incidence for patients who were not upgraded after excision of a benign IDP when other major risk factors were excluded.

Design: With IRB approval, 158 patients with IDP on CNB between 2003 and 2008 were identified in a search of the Anatomic Pathology laboratory information system (CoPath). The radiology and pathology data were reviewed by dedicated breast radiologists and pathologists. Clinical follow-up was obtained from the electronic medical record (Epic).

Results: The indications for CNB were: mass 115 (72.8%); calcifications 33 (20.9%); or both 10 (6.3%). There were 113 (71.5%) ultrasound, 44 (27.8%) stereotactic, and 1 (0.6%) MRI-guided CNB. Excision results were: 97 (61.4%) not upgraded, 11 (6.9%) with BC on excision (8 DCIS, 3 IC), and 4 (2.5%) lacked correlation with the CNB site. Excision reports were unavailable for 46 (29.1%). Excluding cases with BI-RADS 5 imaging or concurrent ipsilateral BC, there were 7 (4.4%) true upgrades: 6 DCIS, 1 low-grade IC (tubular features). Patients who were upgraded, who had AH or LCIS on excision (11 patients) and those with a history of prior or concurrent BC (24 patients) were excluded from further analysis. Follow-up was available for 60/62 of these patients and 7 (11.7%) developed BC after a median of 98 months (range 11-159). There were 3 cases of DCIS (2 ipsilateral, 1 contralateral) and 4 cases of IC (2 ipsilateral, 2 contralateral). Follow-up was available for 30/46 patients without an excision report and no history of BC and after a median of 94 months (range 5-164) none developed BC.

Conclusions: The upgrade rate for benign IDP diagnosed on CNB was 4.4%, similar to recent studies. The cumulative BC incidence for those who had surgery and were not upgraded and who had no history of BC was 11.7% at a median of 8 years. When combined with patients without an excision pathology report, the overall BC incidence was 7.6%. Selection bias for excision and close follow-up could contribute to overestimation of subsequent BC incidence.

268 Neoadjuvant Treatment in Breast Cancer; Relationship to HER2 status including HER2 Copy Number using Brightfield Dual in situ Hybridization

Grace Neville[1], *Fionnuala O'Connell*[2], *Michael Bennett*[3], *Linda Feeley*[4], *Seamus O'Reilly*[5], *Tara Jane Browne*[2]. [1]Cork University Hospital, Cork, [2]Cork University Hospital, Cork, Ireland, [3]Cork University Hospital, Cork City, [4]Cork, [5]Cork University Hospital

Background: Limited and conflicting data is available on HER2 copy number and its potential relationship with pathologic response in the neoadjuvant setting particularly with use of Brightfield Dual in situ Hybridization DNA analysis (DISH).

The aim of our study was to compare the HER2 copy number as assessed by DISH in HER2 positive cases (including IHC positive cases (score 3+) and IHC equivocal cases (score 2+) cases amplified by DISH and to evaluate the relationship with pathologic response using an online tool (MD Anderson Cancer Centre algorithm) to calculate the Residual Cancer Burden (RCB).

Design: Sequential neoadjuvant treated breast cancer cases with available CNB and corresponding excision were identified from a single laboratory over a 62-month period. Patient demographics, surgery type, tumor characteristics including grade and type, hormone profile, HER2 status (IHC and DISH). RCB class was determined for each tumor (PCR, RCB I, RCB II, RCB III). Tumor characteristics and HER2 copy number were further evaluated for the HER2 positive subset.

Results: 130 total patients. Mean age 51.7yrs. 40% wide local excision, 60% mastectomy. Tumor characteristics: invasive ductal carcinoma 82.5%, invasive lobular carcinoma 9.7%, metaplastic carcinoma 2.9%, mixed ductal and lobular carcinoma 2.9%, mucinous carcinoma 1.9%. 60.8% were grade 3. Hormone and/or HER2 status was as follows: 52.9% hormone positive/ HER negative; 19.5% hormone positive/HER2 positive; 14.6% hormone negative/ HER2 positive; 13% triple negative. RCB classes were as follows pCR 14.6%, RCB 1 6.9%, RCB 2 36.9%, RCB 41.5%.

42 of 130 cases were HER2 positive. 34/42 were IHC 3+ and 8/42 were 2+ by IHC and amplified by DISH. 1 of 42 cases was a lobular carcinoma; 41 of 42 were invasive ductal carcinoma, NOS. 33/42 were grade 3; 9/42 were Grade 2, DDISH was performed in 39/42 cases. RCB classes for HER2 positive cases were: PCR (8/42; of these 2 were hormone positive); RCB I (8/42; of these 4 were hormone positive); RCB II (19/42; of these 10 were hormone positive); RCB III (7/52; of these 5 were hormone positive). HER2 copy number for each group was as follows: PCR (range 4.3-21.6); RCB I (range 5.76-22.8); RCB II (5.2-20.3); RCB III (3.33-16.1).<

Conclusions: HER2 positive, hormone negative tumors show the highest frequency of complete or near complete pathologic response to treatment. The study suggests that HER2 copy number does not correlate with degree of response to neoadjuvant therapy in HER2 positive tumors.

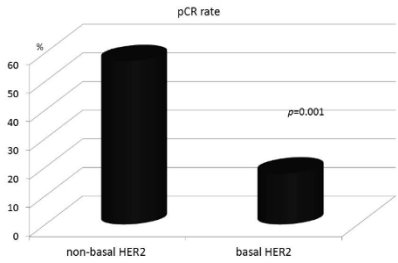
269 Basal Marker Expression Predicts a Lower Pathological Complete Response Rate and Worse Prognosis in ER-Negative/HER2-Positive Breast Cancer Patients with Neoadjuvant Chemotherapy

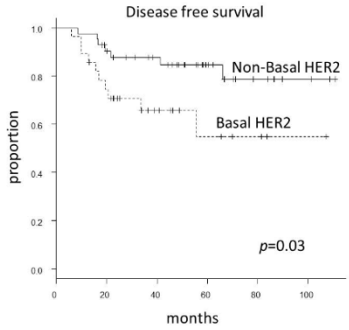
Yasuyo Ohi[1], *Akira I Hida*[2], *Tohru Hayashi*[3], *Yoshihisa Umekita*[4], *Akihide Tanimoto*[2]. [1]Sagara Hosp., Kagoshima City, Kagoshima, [2]Kagoshima University, [3]Brestopia Miyazaki, [4]Tottori University

Background: Almost half cases of ER-/HER2+ patients achieve a pathological complete response (pCR) to neoadjuvant chemotherapy, however, some patients have de novo resistance. Basal HER2 subtype has recently been described and reported to show worse prognosis than non-basal HER2 subtype. Therefore, we explored whether the immunohistochemical expression of basal markers predicts the therapeutic effect of neoadjuvant chemotherapy in ER-/HER2+ breast cancers.

Design: A retrospective cohort study using a clinical database at Sagara and Breastopia Miyazaki Hospital identified 70 eligible patients who received neoadjuvant chemotherapy for stage I-III ER-/HER2+ breast cancers between 2009 and 2015. Basal markers (CK5/6, CK14, EGFR) were immunohistochemically evaluated using pre-therapeutic core needle biopsy samples. Any positive expression of these basal markers was defined as basal HER2 subtype. The pCR (ypT0/Tis, ypN0) rate and disease free survival (DFS) was compared between basal HER2 group and non-basal HER2 group.

Results: Basal HER2 subtype was identified in 40% of ER-/HER2+ breast cancers (28/70). The pCR rate of basal HER2 subtype (17.9%, 5/28) was significantly lower than that of non-basal HER2 subtype (57.1%, 24/42) (p=0.001). According to multivariable analysis, the basal HER2 subtype was an independent predictive factor for lower pCR rate. The DFS of basal HER2 subtype was statistically worse than that of non-basal HER2 subtype (p=0.03).





Conclusions: Basal HER2 subtype, examined by immunohistochemistry, significantly correlates with weak therapeutic response and poor prognosis in ER-/HER2+ breast cancers.

270 A Single Institution Experience with Metaplastic Breast Carcinomas

Andrea C Oliveira[1], *Frederico Ayres*[2], *Cynthia Osorio*[3], *Marcelo Corassa*[2], *Marina De Brot*[4]. [1]A.C.Camargo Cancer Center, São Paulo, São Paulo, [2]A.C.Camargo Cancer Center, [3]A.C.Camargo Cancer Center, São Paulo, Brazil, [4]A.C.Camargo Cancer Center, Sao Paulo, SP

Background: Metaplastic breast carcinoma (MC) constitutes a group of neoplasms characterized by differentiation of the neoplastic epithelium into squamous cells and/or mesenchymal-looking elements. Here, we sought to evaluate clinicopathologic characteristics associated with MC and report outcomes for patients (pts) treated at a cancer center.

Design: A pathology database search was performed to detect pts diagnosed with MC; data were obtained from medical records and pathology reports.

Results: Our search identified 40 pts diagnosed with MC, including 10 squamous cell carcinomas, 2 spindle cell carcinomas, 16 MCs with mesenchymal differentiation, and 7 mixed MCs. Mean age at diagnosis was 49 years (26-91), a positive family history for breast cancer (BC) was noted in 14 pts, and 5 pts had a *BRCA* mutation (2 *BRCA1*, 3 *BRCA2*). Following diagnosis (Dx), 13 pts received neoadjuvant chemotherapy (NCT) with a diverse pathologic response rate (residual cancer burden minimal, 1; moderate, 6; extensive, 4). Of 40 pts, 12 had breast-conserving surgery while 23 underwent mastectomy. Mean tumor size was 3.8 cm, most MCs were categorized as grade 3 (83%) and 9 pts had axillary involvement at Dx. All but 8 MCs showed a triple negative imunophenotype with a mean Ki67 index of 75% and expression of basal markers in 57% of cases, including CK5/6 (45%), CK14 (50%), EGFR (17.5%) and p63 (47.5%). Additionally, MCs exhibited expression of cytokeratins 34betaE12 (12.5%), AE1/AE3 (7.5%), CAM5.2 (22.5%) and 7 (17.5%). Follow-up data were available for 36 pts. Median overall survival was 22 months (mos, range 2-140). Out of 36 pts, 9 (25%) had a local recurrence and 7 (19%) developed distant metastasis to liver, lung, bones, peritoneum, and meninges, among other sites. Of those who recurred, 8 died because of the disease. Survival analyses did not show significant differences for clinicopathologic or immunohistochemical parameters including age, Ki67 index, basal phenotype, family history of BC, patterns of treatment and MC subtype. Of note, basal phenotype showed a marked numeric difference: mOS 17.3 mos for basal vs 52.4 mos for not basal (p=0.05). Size (cut-off 5 cm, (mOS NR vs. 22.3 mo – p=0.009) and lymphovascular invasion (p=0.01) were prognostic.

Conclusions: Our results are in agreement with the current literature that reports MC as a heterogeneous class of neoplasms, with an inferior rate of LN metastases compared to invasive ductal carcinomas of similar size and grade, low response rates to NCT and poor outcome.

271 Can Neovascularization of Mucin Help Distinguish Mucinous Carcinomas from Mucocele-Like Lesions in Breast Core Needle Biopsies?

Allison M Onken[1], *Stuart Schnitt*[2]. [1]Beth Israel Deaconess Medical Center, Boston, MA, [2]Brigham and Women's Hospital; Dana Farber Cancer Institute, Boston, MA

Background: The distinction between mucinous carcinomas (MC) and mucocele-like lesions (MLL), particularly those containing detached epithelial fragments, can be problematic, especially in limited core needle biopsy (CNB) samples. Neovascularization of mucin has been proposed as a criterion to distinguish MC from MLL but its value in helping to categorize mucin-producing breast lesions in CNB has not been previously investigated.

Design: We assessed neovascularization of mucin on hematoxylin and eosin-stained sections of 140 breast CNBs accessioned over a 10-year period (2007-2017) that contained mucin-producing lesions including 52 MC, 71 MLL, and 17 mucin-producing DCIS. MC were further categorized as Capella A (paucicellular), B (hypercellular), or AB (mixed). MLL were further stratified into those with and without epithelial atypia. Neovascularization of mucin was scored as present when thin-walled microvessels were identified within mucin and clearly unassociated with fibrous septae.

Results: Neovascularization of mucin was significantly more frequent in MC than in MLL (69.2% vs 14.1%, p=0.0001). Among MC, mucin neovascularization was significantly more frequent in Capella B and AB lesions (95.5% and 77.8%, respectively) than in Capella A lesions (38.1%) (p=0.00006). However, neovascularization of mucin was still more frequent among paucicellular (Capella A) MC than MLL (38.1% vs 14.1%, p=0.01). Mucin neovascularization was not significantly related to MC size. There was no significant difference in the frequency of mucin neovascularization in MLL with and without atypia (22.2% vs 9.3%, p=0.13). Mucin-producing DCIS less frequently showed neovascularization of mucin than MC (29.4% vs 69.2%, p=0.004); the frequency of mucin neovascularization in mucin-producing DCIS was not significantly different from that in MLL (29.4% vs 14.1%, p=0.16). Sensitivity, specificity, positive predictive value, and negative predictive value of mucin neovascularization for categorizing a lesion as MC were 69.2%, 85.9%, 78.3%, and 79.2%, respectively.

Conclusions: The results of this study indicate that neovascularization of mucin is signficantly more frequent in MC than in MLL in breast CNB. Even paucicellular (Capella A) MC show mucin neovascularization significantly more frequently than MLL. However, neovascularization of mucin was also seen in a small proportion of MLL (14.1%). Therefore, while neovascularization of mucin in a breast CNB favors MC over MLL, its presence is not entirely specific for MC.

272 Are Excisions of Incidental and Imaging-Concordant Lobular Neoplasia Still Justified? Perspective from a Hispanic Cohort

Lurmag Orta[1], *Victor Carlo*[2]. [1]Integrated Pathology Services, Hospital Auxilio Mutuo, San Juan, PR, [2]Integrated Pathology, Trujillo Alto, PR

Background: Current guidelines recommend excision of lobular neoplasia (LN) diagnosed on core needle biopsy (CNB) to evaluate for higher risk lesions. Studies suggest that incidental lesions with pathologic-radiologic concordance can be observed. We studied a cohort of Hispanic patients to evaluate if excisions are justified.

Design: A retrospective review of CNB performed from 01/2012-06/2017 and diagnosed as LN was conducted. LN was defined as lobular carcinoma in situ (LCIS) [classical (c), pleomorphic (p)] and atypical lobular hyperplasia (ALH). Cases with concurrent ipsilateral invasive ductal or lobular carcinoma (IDC, ILC), ductal carcinoma in situ (DCIS), atypical ductal hyperplasia (ADH) or flat epithelial atypia (FEA) were excluded. All available slides and imaging were reviewed.

Results: Of 11290 CNB performed, 132 cases of LN were identified (1.2%) in 129 patients. Excisions were performed in 83 patients (64%), with cLCIS = 20, pLCIS = 2 and ALH = 63; no follow-up was available for the remaining 46. Stereotactic CNB for calcifications were performed in 47 cases (cLN=7, pLCIS=2, ALH=38). Ultrasound-guided CNB for masses were performed in 36 cases (cLN=11, ALH=25). 2 MRI-guided CNB yielded cLCIS. LN was considered an incidental finding in 17 cLCIS and 60 ALH (91%). On excision, upgrades were present in 2 of 68 concordant cases of cLN. In both cases, DCIS was present away from the biopsy site (target area) and are thus considered not true upgrades. A fibroadenoma (FAD) was the target lesion in one of these patients, who had a concurrent contralateral IDC. Of note, 18 FAD associated with LN showed no upgrade on excision. Five excisions showed the following high-risk lesions: 4 ADH and 1 FEA. No residual lesion was identified in 27 cases. Excisions for 17 discordant cases showed 1 IDC (asymmetry) and 1 ILC (suspicious mass), while the remainder showed 1 ADH, 1 FEA, 4 intraductal papillomas, 1 FAD, 4 residual cLN and 4 no residual lesion. For the 2 cases of pLCIS, both were considered the target lesion (suspicious calcifications). One case was upgraded to ILC and 1 case had residual pLCIS.

FOR TABLE DATA, SEE PAGE 122, FIG. 257

Conclusions: LN is an incidental finding in the majority of cases diagnosed on CNB. For cases of cLN and concordant imaging findings, observation can be recommended. Discordant cases had an upgrade rate of 12%, confirming the importance of radiological correlation in assessing the sampling of the target lesion. As pLCIS portends a higher risk of upgrade, excisions should continue to be recommended.

273 Histological Grade Can Be Related to MYB/MYBL1 Gene Rearrangement Patterns in Adenoid Cystic Carcinoma of the Breast

Tomo Osako[1], *Seiji Sakata*[2], *Yuki Togashi*[3], *Yukiko Sato*[1], *Hiroaki Kanda*[4], *Shinji Ohno*[5], *Futoshi Akiyama*[6], *Kengo Takeuchi*[7]. [1]Cancer Institute Hospital, Japanese Foundation for Cancer Research, Koto-ku, Tokyo, [2]Pathology Project for Molecular Targets, Cancer Institute, Japanese Foundation for Cancer Research, [3]Pathology Project for Molecular Targets, Cancer Institute, Japanese Foundation for Cancer Research, Koto, Tokyo, [4]The Cancer Institute of JFCR, Koto-ku Tokyo, Japan, [5]Breast Oncology Center, Cancer Institute Hospital, Japanese Foundation for Cancer Research, [6]Cancer Institute, Japanese Foundation for Cancer Research, [7]Pathology Project for Molecular Targets, CI, JFCR, Tokyo, Japan

Background: Adenoid cystic carcinoma (ACC) is a rare type of triple-negative breast cancer, comprising < 0.1% of breast carcinomas. Breast ACC shows a similar histology to its counterpart of the salivary gland, which is characterized by *MYB-NFIB* or *MYBL1-NFIB*. We aim to elucidate a relationship between morphological features and *MYB/MYBL1* gene rearrangement patterns in breast ACCs.

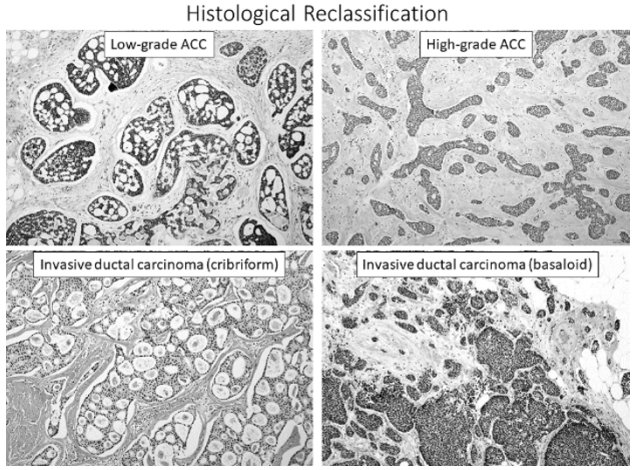
Design: We reviewed 24 cases originally diagnosed as ACC at the Cancer Institute Hospital (Tokyo, Japan) between 1946 and 2016 (0.08% of 29,984 breast carcinoma cases). The histological grade was scored based on the proportion of solid component and presence of high-grade transformation. Cases with solid component < 50% of the tumor area without high-grade transformation were defined as low-grade ACCs, and the other cases as high-grade ACCs.

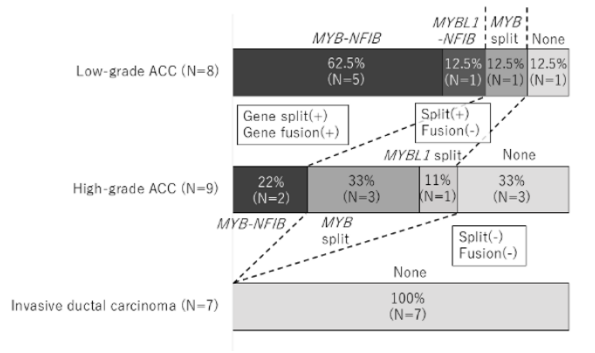
Split fluorescence *in situ* hybridization (FISH) assay for *MYB*, *MYBL1* and *NFIB*, and fusion FISH assay for *MYB-NFIB* and *MYBL1-NFIB* were performed in the 24 cases with our originally-designed probe sets.

Results: Of the 24 originally-diagnosed "ACCs", 8 (33.3%), 9 (37.5%) and 7 tumors (29.2%) were histologically reclassified into low-grade ACC, high-grade ACC, and invasive ductal carcinomas showing cribriform or basaloid histology, respectively (Figure 1, Table). Proportions of the tumors harboring *MYB*/*MYBL1* aberrations were 87.5% (7/8) for low-grade ACCs, 66.7% (6/9) for high-grade ACCs, and 0% for invasive ductal carcinomas (0/7) (Figure 2).

Of the 7 low-grade ACCs harboring *MYB*/*MYBL1* aberrations, 6 showed both *MYB*/*MIBL1* splits and *MYB/MYBL1-NFIB* fusions, and one showed *MYB* split without *MYB-NFIB* fusion or *NFIB* split. Of the 6 high-grade ACC harboring *MYB*/*MYBL1* aberrations, 4 showed *MYB/MBL1* split without *MYB/MYBL1-NFIB* fusion or *NFIB* split, and two showed both *MYB/MIBL1* split and *MYB/MYBL1-NFIB* fusions.

FOR TABLE DATA, SEE PAGE 122, FIG. 273





Conclusions: In the present study, approximately 80% of breast ACCs harbored *MYB/MYBL1* aberrations, whereas invasive ductal carcinomas mimicking ACC did not. Low-grade ACCs were more likely to harbor *MYB/MYBL1* aberrations than high-grade ACCs. Regarding the *MYB/MYBL1* aberration patterns, low-grade ACCs were more likely to harbor *MYB/MYBL1-NFIB* gene fusion, whereas high-grade ACC harbored *MYB/MYBL1* aberrations without fusing to *NFIB*. Thus, histological grade can be related to *MYB/MYBL1* gene rearrangement patterns in breast ACC.

274 Repeating HER2 on Excision Specimens of Invasive Breast Cancer. Is it Enough to Repeat Only on High Grade Carcinomas?

Twisha H Oza[1], *Jessica N Beyda*[2], *Adriana Corben*[2], *Shabnam Jaffer*[3]. [1]The Mount Sinai Hospital, New York, NY, [2]Mount Sinai Hospital, New York, NY, [3]New York, NY

Background: Accurate determination of HER2 status in invasive breast cancer (IC) is essential to offer HER2-targeted therapy. ASCO-CAP 2013 guidelines recommend initial immunohistochemical (IHC) testing on core biopsy (cbx). Excluding HER2 negative (0-1+) and positive (3+) cases on cbx, criteria for repeat testing have been recommended on excisions in specific cases; for e.g., high nuclear grade tumors that are HER2 negative or equivocal (EQ) on cbx. At our institution, all ihc HER2 negative and EQ cases on cbx are repeated on excision, regardless of grade. The purpose of this study was to analyze the validity of our practice, taking into consideration the grade and any specific variant of the IC.

Design: From 1/14 to current, we identified 326 cbx that were either HER2 negative or EQ on IHC that were re- tested on excision. We analyzed the results in relation to the grade and any associated variant. FISH data was also evaluated.

Results: HER2 IHC status changed in 38/326 (11.6%) cases, mostly due to change from HER2 negative to EQ = 34, HER2 negative to positive = 2, and HER2 EQ to positive = 2. The grades of the positive cases were moderate (MD) in 3 and poor (PD) in 1; the latter case was a PD IC with focal mucinous features. The histologic grades for the EQ cases were: well differentiated (WD) = 2, MD = 25 and PD =7. One of the WD IC was tubular and 1 of the MD IC was pure mucinous. Of the PD IC, they were as follows: micropapillary=4, apocrine =1, invasive lobular carcinoma (ILC) with pleomorphic features = 2. Excluding HER2 negative cases, FISH was performed on 1+ and EQ cases (n=186) and converted to positive on either cbx/excision in 11 cases (6%). The histologic grades of these positive FISH cases were: PD=6, MD=5 and were all conventional ductal type.

Conclusions: HER2 IHC remained consistently same from cbx to excision in IC variants such as tubulo-lobular, cribriform, ILC, metaplastic and adenoid cystic, with minor changes noted in tubular, apocrine, mucinous, micropapillary and ILC pleomorphic type. We found 88.4% concordance in HER2 results from cbx to excisions. Discordances were mostly due to change to EQ (10.4%), with few changes to positive (1.2%), due to sampling and heterogeneity. Most of the cases that converted to EQ/positive on IHC and FISH were MD (10.4%), followed by PD (4%). WD IC converted to EQ in only 0.6%, with no change to positive noted. Thus, while we agree with retesting of PD IC on cbx with negative/EQ HER2 IHC, testing of MD IC should be re-considered.

275 Is it Necessary to Measure Invasive Breast Carcinoma on Core Biopsy? - An Analysis of Ultrasound, Mammotome, and MRI Guided Core Biopsies.

Twisha H Oza[1], *Jessica N Beyda*[2], *Adriana Corben*[2], *Shabnam Jaffer*[3]. [1]The Mount Sinai Hospital, NY, New York, NY, [2]Mount Sinai Hospital New York, New York, NY, [3]New York, NY

Background: Invasive breast cancer (IC) pathological size is an established strong prognostic factor and thus should be measured carefully. With increased screening, most invasive cancers (IC) are initially diagnosed on core biopsy (cbx), particularly small ones. This has created an impetus to measure IC on cbx. The goal of our study was to evaluate the validity of this practice, particularly in relation to the cbx technique and intactness (or not) of IC on cbx.

Design: Using the pathology database, we identified 148 cases of IC on cbx and excision performed at our institution from 1/2014 to current. The greatest linear extent of IC was measured on both cbx and excision and was correlated with the gross and imaging when available. Of these cases, we separately identified and analyzed a subset of 85 cases with intact IC on cbx, i.e., continuous tumor with no broken edges, for better correlation.

Results: See Table below.

Table: Correlation of technique of cbx, intactness of cbx, histologic type and pathological stage

Needle biopsy (all)	Comparison of pathological stage (cbx vs excision) n=148 (all)		
	Upstaged, 77 (52%)	Downstaged, 16 (11%)	No change, 55 (37%)
US=88	47 /88(53%)	7/88 (8%)	34/88 (39%)
Mammo=37	24/37 (65%)	4/37(11%)(3 no residual)	9/37 (24%)
MRI=23	6/23(26%)	5/23(22%)(1 no residual)	12/23 (52%)
Histologic type			
Lobular carcinoma= 22	11/22 (50%)	9/22 (40%)	2/22 (9%)
Needle biopsy (intact cbx)=85	Comparison of pathological stage (cbx vs excision) n=85 (intact cbx)		
	Upstaged, 43/85 (51%)	Downstaged,11/85 (13%)	No change, 31/85 (36%)
US=44	22/44 (50%)	3/44 (7%)	19/44(43%)
Mammo=27	16/27 (60%)	4/27(15%)	7/27(25%)
MRI=14	5/14(35%)	4/14 (30%)	5/14(35%)

Conclusions: Approximately half of all IC on cbx upstaged on excision, whereas the remaining half either downstaged or remained the same, (no residual IC on 4 T1a cases). A higher upstage was seen with mammo cbx compared to other types of cbx. This is due to better correlation with US detected masses (as seen in all US bx that down staged or remained same on excision) vs incidental mammo detected IC during work up of Ca++. All downstages occurred in IC <1cm, and most (80%) upstages in >1cm, predominantly within 1 tumor stage denomination difference. The presence of intact linear tumor did not improve correlation of size between cbx and excision. There was no difference in correlation between ductal and lobular phenotype. It is prudent to measure IC on both cbx and excision, especially in IC < 1cm, and correlate with imaging studies, so as to best determine greatest IC size for optimal patient treatment.

276 An Appraisal of Metaplastic Carcinoma Denoted as Such by Squamous Differentiation

Andrea Pannunzio[1], *J. Jordi Rowe*[2], *Olaronke Oshilaja*[2], *Charles Sturgis*[3], *H. Evin Gulbahce*[4], *Christine Booth*[5], *Erinn Downs*[6]. [1]Cleveland Clinic, Cleveland, OH, [2]Cleveland Clinic, Cleveland, OH, [3]Cleveland Clinic, Cleveland, OH, [4]University of Utah, Salt Lake City, UT, [5]Cleveland Clinic, Cleveland, OH, [6]Cleveland Clinic, Cleveland, OH

Background: Currently no established criteria exist defining what extent of metaplasia within invasive mammary carcinoma is required to garner a designation as metaplastic carcinoma (MC). With squamous metaplasia/differentiation (SM/D), the metaplastic component may consist of scattered microscopic foci or compose 100% of the tumor (so called squamous cell carcinoma of the breast). Studies have shown that squamous cell carcinoma (SCC) of the breast is an aggressive variant, however the behavior of those carcinomas with lesser degrees of SM/D have not been well studied. We recorded extent of SM/D in a series of primary invasive mammary carcinomas (IMC) and assessed the impact on locoregional recurrence (LR) and distant metastases (DM).

Design: After IRB approval, primary IMC indexed as invasive carcinoma with SM/D or SSC were reviewed, recording extent of SM/D of the total tumor volume. When nodal disease or metastatic disease was present, available slides were scored for SM/D. Patient age, date of diagnosis, estrogen receptor (ER), progesterone receptor (PgR) and HER2 status were obtained from chart review. Clinical follow up was obtained through the institutional tumor registry. Recurrence was analyzed using logistic regression.

Results: 46 patients were identified for inclusion, all patients were female ranging from 34-95 years of age. All tumors were Nottingham grade 3, 11 (24%) were ER + and 4 (8%) PgR+. HER2 status was unknown for 2, with HER2 amplification present in 13/44 (30%). 25 (57%) were triple negative. SM/D composed from 5% to 100% of the tumor volume (median 20%, with 16 cases having 40% or > SM/D). In the HER2 amplified population, SM/D ranged from 5-80% (median 20%). Of the 17 (40%) cases with nodal disease, 12 had slides for review with 9 (75%) having SM/D (range 5-90%). Of the 13 (28%) cases with DM disease, 7 had slides for review with all 7 having SM/D (range 5-40%). LR was seen in 4 patients with a mean follow up of 33 months and DM disease was seen in 13 patients with a mean follow up of 45 months. There was no correlation between the extent of SM/D and LR or DM.

Conclusions: Given the dogma that most MC are triple negative, the HER2+ seen in 28% and ER+ in 24% raises the possibility that SM/D does not equate to MC in the traditional sense. A matched control study is warranted to assess if SM/D is correlated with differences in disease free and overall survival. Noting the presence of SM/D in the primary is useful as the nodal disease and DM frequently have SM/D.

277 Pleomorphic Adenoma and Mucoepidermoid Carcinoma of the Breast Are Underpinned by Fusion Genes

Fresia Pareja[1], *Anqi Li*[2], *Hannah Y Wen*[3], *Felipe Geyer*[4], *Rodrigo Gularte-Merida*[5], *Rahul Kumar*[6], *Gouri J Nanjangud*[6], *Nora Katabi*[6], *Zsuzsanna Varga*[7], *Emad Rakha*[8], *Edi Brogi*[3], *Britta Weigelt*[3], *Jorge Reis-Filho*[9]. [1]MSKCC, New York, NY, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]Memorial Sloan Kettering Cancer Center, New York, NY, [4]Memorial Sloan-Kettering Cancer Center, New York, NY, [5]Memorial Sloan Kettering Cancer Center, [6]MSKCC, New York, NY, [7]University Hospital, Zurich, Switzerland, [8]University of Nottingham, Nottingham, England, [9]Memorial Sloan-Kettering CC, New York, NY

Background: Salivary gland-like tumors of the breast histologically resemble their salivary gland counterparts. Whilst some of these tumors are underpinned by the same molecular alterations both in the breast and in the salivary glands (e.g. adenoid cystic carcinomas (*MYB*/*MYBL1* rearrangements) and secretory carcinomas (*ETV6-NTRK3* fusion gene)), others have distinct molecular profiles according to the anatomical site (e.g. acinic cell carcinoma). Here we sought to define whether breast pleomorphic adenoma (PA) and mucoepidermoid carcinoma (MEC) harbor the same molecular alterations (*PLAG1*/*HMGA2* and *MAML2* rearrangements, respectively) as their salivary gland counterparts.

Design: Four PAs and one MEC which were reviewed and classified according to World Health Organization criteria. Three PAs and 1 MEC were subjected to RNA sequencing; fusion genes were detected using benchmarked bioinformatics algorithms, and validated by RT-PCR and/or FISH using *PLAG1* and *HMGA2* break-apart probes and *CRTC1-MAML2* dual fusion probe. One PA with insufficient material for RNA sequencing was interrogated for the presence of *PLAG1* and *HMGA2* rearrangements by FISH. *In vitro* functional studies were performed utilizing non-malignant breast epithelial cells (i.e., MCF-10A and MCF-12A) in which *HMGA2-WIF1* or *CRTC1-MAML2* fusion genes were introduced using CRISPR-Cas9.

Results: Three cases (2/4 PAs and 1/1 MEC) harbored the fusion genes reported in the respective salivary gland counterpart. RNA sequencing of a PA revealed a *HMGA2-WIF1* fusion gene, and another PA harbored a *PLAG1* rearrangement detected by FISH. The MEC harbored a *CRTC1-MAML2* fusion gene detected by RNA sequencing, which was confirmed by FISH. Expression of the *HMGA2-WIF1* or the *CRTC1-MAML2* fusion genes in MCF-10A and MCF-12A cells resulted in increased cellular proliferation and phenotypic changes, with enlarged multi-acinar structures in three-dimensional organotypic cell cultures.

Conclusions: PAs and MECs arising in the breast harbor the fusion genes described in their salivary gland counterparts, constituting additional examples of genotypic-phenotypic correlations regardless of the anatomic site. *HMGA2-WIF1* and *CRTC1-MAML2* fusion genes result in oncogenic transformation in *in vitro* mammary cellular models. *PLAG1*/*HMGA2* or *MAML2* rearrangements may be used as confirmatory diagnostic markers of breast PAs and MECs, respectively. The drivers of breast PAs and MECs lacking the respective fusion genes have yet to be identified.

278 MicroRNA-137 and -496 Additively Inhibit Cell Migration and Invasion by Targeting DEL-1 in Triple Negative Breast Cancer Cells

Ji-Young Park[1], *Ji Yun Jeong*[2], *Sun Young Kwon*[3], *Eun Hye Lee*[4], *An Na Seo*[5], *Jin Hyang Jung*[4]. [1]Kyungpook National University School of Medicine, Daegu, Daegu, [2], Daegu, Korea, [3]Keimyung Univ/Dongsan Medical Center, Daegu, [4]Kyungpook National University School of Medicine, [5]Daegu

Background: Although Del-1 was recently proposed as a new biomarker for breast cancer in our previous studies, the mechanisms of Del-1 expression are barely understood. As miRNAs are widely accepted to play a crucial role in expression of target genes, we selected two microRNAs (miR-137 and -496) potentially affecting Del-1 expression and examined their impact on Del-1 expression in a variety of breast cancer cells and tissues.

Design: Del-1 mRNA and microRNA levels were measured by qRT-PCR among breast cancer cells (MDA-MB-231, MCF7, SK-BR3 and T-47D) and tissues from 10 patients with triple-negative (TN) breast cancer. The effects of microRNAs on cell proliferation and invasion were detected using MTT, wound healing, and Transwell assays. Furthermore, luciferase reporter assay was used to identify the direct regulation of Del-1 by miR-137 or miR-496 in MDA-MB-231 cells.

Results: As both miR-137 and -496 levels were low in all breast cancer cell lines and TN tissues but Del-1 mRNA expression was remarkably higher in MDA-MB-231 compared to the other breast cancer cell lines, further functional analyses were done with MDA-MB-231 representing triple-negative breast cancer subtype. Both miR-137 and miR-496 were respectively revealed by luciferase reporter assay to directly bind at the 3'-UTR of Del-1 and moreover Del-1 expression was upregulated by inhibitors and reversed by mimics of both miR-137 and miR-496. As shown in previous studies about Del-1 effect on breast cancer, both microRNAs also inhibited cell proliferation, migration and invasion of MDA-MB-231 with additive pattern. These result suggests that miR-137 and miR-496 affect cancer progression via alteration of Del-1 expression.

Conclusions: Although Del-1 was recently introduced as a new biomarker for triple negative breast cancer, the mechanisms of Del-1 expression were barely identified. The current study firstly demonstrated that microRNA-137 and -496 are involved in Del-1 regulation by binding at Del-1 gene, affecting cancer progression by altering Del-1 expression.

279 Del-1 Expression in Triple-Negative Early Breast Cancer

Ji-Young Park[1], *Ji Yun Jeong*[2], *Eun Hye Lee*[3], *Jin Hyang Jung*[3]. [1]Kyungpook National University School of Medicine, Daegu, Daegu, [2]Daegu, Korea, [3]Kyungpook National University School of Medicine

Background: While a differential diagnostic role for plasma del-1 in early breast cancer was proposed in our previous study, this study excluded the possible impact of endothelial-derived del-1 by examining the tumoral expression of del-1 in breast cancer cell lines and tumor specimens and then analyzed the prognostic impact in patients with early breast cancer (EBC).

Design: The del-1 mRNA expression was assessed in breast epithelial (MCF10A) and cancer cells (MDA-MB-231, MCF7, SK-BR3, and T-47D) using a real-time PCR. Meanwhile, the tumoral expression of del-1 was determined based on tissue microarrays and immunohistochemistry (IHC) from a consecutive series of 440 EBC (stage I-IIIA) patients who underwent surgery between 2011 and 2013. The del-1 expression was scored according to the staining intensity of positive tumor cells (0 through 3) and followed by an association analysis with the clinical/pathological characteristics and outcomes of the EBC patients.

Results: While a high del-1 mRNA expression was found in all the breast cancer cell lines, the expression was significantly higher in MDA-MB-231. The tumoral expression of del-1 was also significantly associated with a negative expression of ER or PR, and low expression of Ki-67, all of which were connected to a high expression and intensity of del-1, particularly in the case of triple-negative EBC and regardless of the stage (100%, 98.5%, and 95.7% for triple negative, HER2 overexpressing, and hormone responsive subtypes, respectively; $P<0.036$). Plus, a correlation was found between del-1 expression and an aggressive histologic grade, particularly nuclear mitosis and polymorphism, suggesting a possible role in tumor progression. However, no association was found between del-1 expression and the tumor burden, such as the tumor size and nodal metastasis. In the survival analysis, a worse distant disease-free survival trend was noted for patients with tumoral del-1 overexpression, particularly in the node-positive subgroup.

Conclusions: While all the investigated breast cancer cell lines exhibited del-1 expression, the expression rate and intensity were specifically prominent in the triple-negative subtype, suggesting del-1 as a new diagnostic marker. Plus, based on the relationship with an unfavorable histology and worse survival trend, tumor or plasma del-1 could act as a molecular target in patients with triple-negative breast cancer.

280 Non-hematological Secondary Tumors in Breast from Extramammary sites: Study of 39 cases

Asawari Patil[1], *Divya Kannan*[2], *Akash P Sali*[3], *Tanvee S Kulkarni*[4], *Sangeeta Desai*[5], *Tanuja Shet*[6]. [1]Tata Memorial Centre, Mumbai, Maharashtra, India, [2]Tata Memorial Centre, Mumbai, India, [3]Tata Memorial Hospital, Mumbai, Maharashtra, [4]Tata Memorial Centre,

Mumbai, Maharashtra, [5]Tata Memorial Centre, Mumbai, [6]Tata Memorial Hospital, Mumbai, Maharashtra, India

Background: Metastatic tumors in breast from extramammary sites are rare (0.5-2% of breast malignancies). Differentiating these tumors from primary breast malignancies can be difficult clinically and histologically. The aim of present study was to review clinicopathological features of metastatic tumors in breast diagnosed at our institute.

Design: Cases with histological/cytological diagnosis of metastasis to breast from January 2004 to August 2017 were identified. Histopathological features were reviewed. Clinical details were obtained from electronic medical records.

Results: Total 39 cases were identified (all females, age range 16-85 yrs, median – 54 yrs). Thirty-eight cases were diagnosed on histology, while diagnosis was made on FNAC in one case. Commonest diagnosis was carcinoma (n= 25) including Adenocarcinoma (n=20), Neuroendocrine tumor (n=3; 2 high grade neuroendocrine carcinoma, 1 atypical carcinoid), Medullary thyroid carcinoma (n=1), Sarcomatoid carcinoma (n=1). Melanoma was second common diagnosis (n=7), followed by Sarcoma (n=6, 3 high grade sarcoma, 2 rhabdomyosarcoma/RMS, 1 osteogenic sarcoma/OGS, 1 alveolar soft part sarcoma/ASPS). Single breast was involved in 34 cases, of which 28 were unifocal. Five showed bilateral, multifocal involvement. Primary site was known in 27 cases at the time of presentation, while it was identified in 6 more cases over the course of patient management. The primary site remained unknown in 6 cases (5 melanomas, 1 atypical carcinoid). Most common primary site was ovary (n=13, all adenocarcinoma), followed by extremities (n=7, 2 melanomas, 2 high grade sarcomas, 1 RMS, 1 OGS, 1 ASPS) and lung (n=3; 2 adenocarcinoma, 1 neuroendocrine carcinoma). Other sites included stomach (n=2), orbit (n=1), thyroid (n=1), esophagus (n=1), endometrium (n=1), peritoneum (n=1), rectum (n=1), oral cavity (n=1) and pancreas (n=1). Majority cases were diagnosed on biopsy (n=28) and one case on FNAC. Morphology and immunoprofile, assisted by clinico-radiological inputs was of prime importance for accurate diagnosis in these cases. Of the remaining cases, 9 underwent upfront lumpectomy while upfront modified radical mastectomy was done in one case.

Conclusions: Differentiating metastatic tumor in breast from primary breast malignancy is essential for optimum therapy and to avoid unnecessary radical surgery. Pathologists need to be aware of this rare entity. Unusual histopathological features and clinico-radiological findings can provide important clues for correct diagnosis.

281 The Economics of an Academic Breast Pathology Service

Jessica Peak Vanleer[1], *Steven Johnson*[2], *Siobhan M O'Connor*[3], *Susan Maygarden*[4]. [1]University of North Carolina Hospitals, Chapel Hill, NC, [2]University of North Carolina School of Medicine, Chapel Hill, NC, [3]University of North Carolina, Chapel Hill, NC, [4]Univ. of North Carolina, Chapel Hill, NC

Background: Many pathologists share the perception that breast pathology specimens are undervalued by CPT codes for the amount of work required to appropriately evaluate these cases. The aim of this study is to determine whether this perception is supported by objective data through quantification and comparison of work associated with breast specimens versus non-breast specimens.

Design: All in-house breast cases received over four-2.5 week time periods spanning 11 months in 2016-2017 were reviewed. Only cases with 88305, 88307, or 88309 charges were analyzed. 402 breast cases consisting of 911 total specimens were identified and divided into 88305 (n=580), 88307 (n=320), and 88309 (n=11) charges. The comparison group consisted of consecutive non-breast cases collected over the same time periods with an equal number of billing charges. For each specimen, data measures included specimen type, initial hematoxylin and eosin stained sections (H&Es), pre-ordered additional H&E stained sections (levels), and additional H&E stained sections ordered after initial slide review (recuts). Data measures for breast and non-breast specimens were compared charge for charge. Data was analyzed by chi-square tests and a p-value <0.05 was considered statistically significant.

Results: For 88305 charges, breast specimens consisted mostly of core needle biopsies and margins (81%), and non-breast specimens were primarily gastrointestinal biopsies (64%). Breast 88305 specimens generated 1.52 times the total number of slides than did non-breast specimens (p=0.18, Table 1). For 88307 charges, breast specimens consisted mostly of lymph nodes and lumpectomies (69%), and non-breast specimens were evenly distributed across organ systems. Breast 88307 specimens generated 1.84 times the total slides than did non-breast specimens (**p=0.02**). While our 88309 cohort was underpowered (n=11), we found that the breast specimens generated 2.08 times the total slides than non-breast specimens (p=0.41). When comparing all charges (n=911), breast specimens generated 1.84 times the initial H&Es (**p=0.01**) and 1.71 times the total slides (**p=0.01**) than non-breast specimens.

	88305 (n=580)				88307 (n= 320)			
	Breast (B)*	Non-Breast (NB)*	B/NB Ratio	p	Breast (B)*	Non-Breast (NB)*	B/NB Ratio	p
Initial H&Es	2.69	1.17	2.29	0.06	9.07	5.47	1.66	0.08
Levels	1.20	1.58	0.76	0.58	1.53	0.43	3.60	0.16
Recuts	0.36	0.06	6.39	0.26	0.62	0.22	2.78	0.43
Total Slides	4.26	2.81	1.52	0.18	11.22	6.11	1.84	0.02

	88309 (n=11)				Total (n=911)			
	Breast (B)*	Non-Breast (NB)*	B/NB Ratio	p	Breast (B)*	Non-Breast (NB)*	B/NB Ratio	p
Initial H&Es	23.64	12.27	1.93	0.49	5.18	2.81	1.84	0.01
Levels	0	0	0	0	1.30	1.60	1.13	0.78
Recuts	3.64	0.82	4.44	0.66	0.49	0.12	3.97	0.15
Total Slides	27.27	13.09	2.08	0.41	6.98	4.09	1.71	0.01

* Values represent average number of initial H&Es, levels, recuts and total slides per billing charge

Conclusions: Work performed on breast biopsies (88305) may be comparable to non-breast biopsy cases. However, regarding breast cancer resections (88307 and 88309), nearly twice as many slides are generated as compared to similarly coded non-breast cases. These findings support the concept that CPT codes need to be revised to better reflect the work associated with breast cases.

282 AJCC Eight Edition Invasive Breast Cancer Staging; Reappraisal of the Manual and Assessment of Impact

Mirna B Podoll[1], *Kamaljeet Singh*[2], *David Edmonson*[3], *C. James Sung*[4]. [1]The Warren Alpert Medical School of Brown University, Providence, RI, [2]Sharon, MA, [3]Women & Infants Hospital, [4]Women & Infants Hospital/Brown University, Providence, RI

Background: In the eighth edition of the AJCC breast cancer staging manual, in addition to TNM stage (anatomic stage), the overall prognostic stage is computed using the histologic grade, Oncotype Dx (ODx) results, hormone receptor (estrogen and progesterone) and HER2 status. This novel staging is based on a previously described "bioscore-based" staging system. We aimed to study the impact of the new prognostic staging.

Design: Consecutive invasive breast carcinomas treated at one institution (01/2007-12/2016) were identified. Cases that underwent neoadjuvant therapy and cases with incomplete information for calculating the prognostic stage were excluded from the study. Clinicopathologic features including the disease specific (overall and recurrence free) survival were obtained from the cancer registry. Prognostic stage was computed using AJCC 8th edi. manual (Springer, 2017). All cases were also stratified into bioscore groups (0-7), using previously described method (Mittendorf E Ann Surg Oncol 2017). Stage distribution by new and old staging was compared. Survival was estimated by the Kaplan-Meier estimator (SPSS ver. 24) for the new staging and bioscore.

Results: Total of 1766 cases qualified for the study. Staging criteria were not identified for 99 (5.6%) cases in the new manual. Prognostic and anatomic stage differed in 800 (45.3%) cases: 453 (25.7%) upstaged and 347 (19.6%) downstaged. ODx testing was performed in 313 (17.7%) cases, which lowered stage in 62 (3.5%) cases. Stage 3A and 1A cases underwent highest and lowest % stage change respectively (3A >2B>2A>1B>3C>3B>1A). Both prognostic staging and bioscore-based groups identified distinct overall survival groups (^{2}=65 Vs. 62; Log-Rank) and performed better than older staging (7th Edi.). Bioscoring system also performed better in estimating recurrence free survival (χ^2= 113 Vs. 97; Log-Rank).

	1A	1B	2A	2B	3A	3B	3C	N (7th Edi.), n (%)
1A	780 (72)	196 (18)*	110 (10)*					1086 (65)
1B	41 (65)^	17 (27)	4 (6)*	1 (2)*				63 (4)
2A	9 (3)^	202 (60)^	46 (14)	33 (10)*	49 (15)*			339 (20)
2B	1 (2)^	20 (37)^	0	4 (7)	20 (37)*	3 (6)*	6 (11)*	54 (3)
3A		6 (7)^	2 (3)^	47 (58)^	2(3)	18 (22)*	6 (7)*	81 (5)
3B						6 (46)	7 (54)*	13 (1)
3C					2 (7)^	17 (55)^	12 (39)	31 (2)
Total (8th Edi.), n (%)	831 (50)	441 (28)	162 (10)	85 (5)	73 (4)	44 (3)	31 (2)	1667 (100)

*upstage, ^downstage & shaded cells- no change in stage

Recurrence Free Survival (months)				Overall Survival (months)			
Bioscore	Mean	Lower bound	Upper bound	Bioscore	Mean	Lower bound	Upper bound
0	83.6	81.4	85.8	0 & 1*	87.0	86.6	87.5
1	86.4	85.8	87.1				
2	85.6	84.8	86.4	2	85.1	84.1	86.1
3	83.7	81.4	86.1	3	86.1	84.5	87.7
4	81.9	78.3	85.5	4	82.7	79.3	86.0
5	73.0	63.8	82.2	5	80.4	73.3	87.5
6	53.0	42.8	63.1	6 & 7*	49.7	39.6	59.8
7	23.5	10.4	36.5				
Overall	86.6	85.9	87.3	Overall	87.5	87.0	88.1

*Bioscores 0 & 1 and 6 & 7 combined for overall survival

Conclusions: The new staging guidelines improve the overall and disease free survival estimates. Prognostic staging scheme changed the final stage in ~50% of cases with slightly higher upgrades than downgrades. Future editions may include all possible combinations of the prognostic stage components.

283 CTLA-4 Expression in Tumor Infiltrating Lymphocytes in Pregnancy Associated Breast Cancer

Lauren Rosen[1], *Jorge Novo*[2], *Julianne M Ubago*[3], *Jennifer Pincus*[4], *K. P. Siziopikou*[3], *Luis Blanco*[5]. [1]Chicago, IL, [2]Northwestern University Feinberg School of Medicine, Chicago, IL, [3]Northwestern University, Chicago, IL, [4]Northwestern Memorial Hospital, Chicago, IL, [5]Northwestern University Feinberg School of Medicine, Chicago, IL

Background: Pregnancy associated breast cancer (PABC) is typically triple negative and is associated with a poor prognosis. We previously assessed the immune microenvironment of invasive breast carcinomas in young women and reported that PABC has more prominent tumor infiltrating lymphocytes (TILs), with significant expression of PD-L1. CTLA-4 is a critical immune checkpoint marker, which serves as a negative regulator of T cell response, and emerging evidence suggests an important role for CTLA-4 in breast cancer progression. Targeted therapies are available for both CTLA-4 and PD-L1, with studies showing that combination therapy offers improved benefit by inhibition of both pathways. In this study, we assessed expression of CTLA-4 in both TILs and tumor cells in PABC and in age-/stage-/grade-matched nulliparous women, and correlated their expression with clinicopathologic characteristics, including PD-1 and PD-L1 expression.

Design: We evaluated 21 patients diagnosed with PABC within 2 years of pregnancy (mean age 35.7, range 26-48) and 14 matched controls (mean age 37.5, range 29-51). Slides were reviewed and the pathologic tumor characteristics including TILs, were noted. The extent and intensity of CTLA-4 immunoreactivity was assessed, and a composite score (CS) was calculated by multiplying extent by intensity.

Results: CTLA-4 TILs expression was similar in PABC (16/21 cases, 76%, mean CS=1.48) and controls (8/14 cases, 57%, mean CS=1.21). Expression of CTLA-4 by TILs was independent of tumor grade, hormone receptor and HER2 status, and other histologic features including lymph node metastases. All 16 cases with TIL expression of CTLA-4 were also positive for PD-L1 and PD-1. Immunoreactivity in the tumor cells was rare with only 3 PABC and 4 controls expressing CTLA-4.

Conclusions: 1. CTLA-4 expression by TILs is frequent in invasive breast carcinomas in young women, and is expressed similarly in TILs in both PABC and controls. **2**. CTLA-4 expression in TILs was independent of tumor characteristics in this series. 4. Rare cases may have CTLA-4 expression in the tumor cells. **3**. All cases with CTLA-4 expression by TILs also expressed PD-1 and PD-L1. 4. All cases with CTLA-4 expression in TILs also expressed PD-1 and PD-L1. Our findings raise the possibility that immune-based therapeutic strategies targeting the PD-1/PD-L1 pathway and CTLA-4 may offer benefit in breast cancer in young women, including PABC, with otherwise limited therapeutic options.

284 The Immune Microenvironment of HER2 Positive Breast Carcinomas; CTLA-4 Expression and the PD-1/PD-L1 Axis

Lauren Rosen[1], *Jorge Novo*[2], *Julianne M Ubago*[3], *Jennifer Pincus*[4], *Luis Blanco*[5], *K. P. Siziopikou*[3]. [1]Chicago, IL, [2]Northwestern University Feinberg School of Medicine, Chicago, IL, [3]Northwestern University, Chicago, IL, [4]Northwestern Memorial Hospital, Chicago, IL, [5]Northwestern University Feinberg School of Medicine, Chicago, IL

Background: Recent studies have highlighted the important role of the immune microenvironment in many human tumors including breast cancer. Emerging data suggest that targeting the PD-1/PD-L1 axis and the CTLA-4 pathway may represent new therapeutic strategies in aggressive tumor subtypes of breast carcinoma. HER2 amplification is found in about 20% of breast carcinomas and is associated with a poor prognosis despite initially effective anti-HER2 therapies. In addition to the classic HER2+ subtype, the Luminal B subtype is another group of breast tumors that could benefit from new therapeutic options. In this study, we evaluated expression of CTLA-4 in both tumor cells and tumor infiltrating lymphocytes (TILs) in classic HER2+ and Luminal B subtypes of breast carcinoma, and correlated expression of CTLA-4 with PD-1 and PD-L1.

Design: The study population consisted of 101 patients with HER2+ invasive breast carcinoma diagnosed from 2009-2014 (mean age 54, range 23-80). Tissue microarrays were constructed (3 cores/case) to account for tumor heterogeneity, and were immunostained for CTLA-4. A composite score (CS) was calculated by multiplying extent by intensity of CTLA-4. The CS for CTLA-4 was then compared to previous CS obtained for PD-1 and PD-L1.

Results: Overall, CTLA-4 was positive in TILs in 27/101 (27%) cases. Expression of CTLA-4 by TILs was higher in HER2+ tumors (18/51, 35%) compared to Luminal B tumors (9/50, 18%) (p=0.04). Approximately 40% (11/27) of cases with CTLA-4 positive TILs were also positive for PD-L1, and 63% (17/27) for PD-1. Of interest, compared with CTLA-4 negative cases, cases with CTLA-4 positive TILs were more often grade 3 (22/27, 81% vs 52/74, 70%). Both had a similar number of lymph node metastases (38% vs 41%). As a group, tumor cells were positive for CTLA-4 in 12/101 (12%) cases; of those 8/51 (16%) were HER2+ tumors and 4/50 (8%) were Luminal B tumors.

Conclusions: 1. CTLA-4 is expressed in TILs in over a third of HER2+ tumors and almost a fifth of Luminal B tumors. **2**. Tumor cell expression of CTLA-4 is seen in 16% of all HER2+ tumors, and a minority of Luminal B tumors. **3**. Almost half of CTLA-4 positive TILs were also positive for PD-L1, and almost two-thirds for PD-1. Our findings add to the understanding of the role of the host/tumor microenvironment in the classic HER2+ and Luminal B subtypes of breast cancer and raise the possibility that single or dual checkpoint blocking therapeutic strategies may show benefit against these aggressive breast carcinomas.

285 Clinicopathologic Features of Unexpectedly HER2 Positive Breast Carcinomas

Lauren Rosen[1], *Jorge Novo*[2], *Luis Blanco*[3], *K. P. Siziopikou*[4], *Jennifer Pincus*[5]. [1]Chicago, IL, [2]Northwestern University Feinberg School of Medicine, Chicago, IL, [3]Northwestern University Feinberg School of Medicine, Chicago, IL, [4]Northwestern University, Chicago, IL, [5]Northwestern Memorial Hospital, Chicago, IL

Background: HER2 is amplified or overexpressed in approximately 12-20% of cases of invasive breast carcinoma and HER2 testing is standard of care, providing both prognostic and predictive information. In a small subset of grade 1 or grade 2 carcinomas with special features (other than ductal carcinoma of no special type), HER2 is unexpectedly positive. In this study, we evaluated the clinicopathologic features in these unexpectedly HER2 positive (HER2+) cases.

Design: The pathology database was searched for cases of invasive breast carcinoma diagnosed on biopsy from 2014 to 2017 to identify HER2+ cases by IHC and/or FISH. Tumors with either a grade 1 or grade 2 with special subtype features usually negative for HER2 were included. Clinicopathologic data collected included age, tumor grade, tumor size, hormone receptor status, lymph node status, and presence of recurrence or metastasis.

Results: A total of 215 HER2+ cases were identified, including 20 tumors that met criteria for our study (9%). Of the 20, 12 tumors were grade 1 (60%) and 8 were grade 2 (40%), comprised of 13 ductal and 7 lobular carcinomas. Of the 19 slides available for review, the majority had a tubule score of 2-3 (19/19), nuclear score of 2-3 (15/19), and mitosis score of 1 (17/19). The average tumor size was 1.8 cm. Four tumors had mucinous features and 1 had micropapillary features. Thirteen (65%) cases were HER2 score 3+ by IHC, 7 were score 2+ by IHC (35%) and reflex FISH testing showed 6 cases were amplified, and 1 was equivocal. The equivocal case was HER2 FISH amplified on excision. The majority of cases were luminal B (18, 90%) and the remaining were HER2 subtype by IHC (2, 10%). Lymph node metastases were present in 5 tumors, all of luminal B subtype. A recurrence occurred in 1 case. The frequency of recurrence was 5% at an average of 109 months. No distant metastases were recorded.

Conclusions: Despite the low frequency of HER2+ well-differentiated breast carcinomas at our institution (5.5%) and in the literature (0.3 - 6.2%), they are important to identify for prognostic information and treatment options. Our population of HER2+ carcinomas showed a similar rate of lymph node metastases and recurrence as poorly-differentiated HER2+ carcinomas, providing additional support that HER2+ indicates a worse prognosis irrespective of grade or special subtype features. These findings additionally support that these tumors will preferentially behave according to their HER2 status and should be regarded and treated in this manner.

286 CDH1 Mutated and CDH1 Wild Type Classic and Pleomorphic Invasive Lobular Breast Carcinomas Differ in Genomic Signatures and Opportunities for Targeted and Immunotherapies

Jeffrey S Ross[1], *Jon Chung*[2], *Julia A Elvin*[3], *Jo-Anne Vergilio*[4], *Shakti Ramkissoon*[5], *James Suh*[6], *Eric Severson*[7], *Sugganth Daniel*[2], *Garrett M Frampton*[2], *Ryan Hartmaier*[2], *Lee Albacker*[1], *Alexa B Schrock*[8], *Siraj Ali*[9], *Vincent A Miller*[2], *Philip M Stephens*[2], *Laurie Gay.* [1]Foundation Medicine, Cambridge, MA, [2]Foundation Medicine, [3]Foundation Medicine, Inc, Cambridge, MA, [4]Foundation Medicine, Inc, Cambridge, MA, [5]Foundation Medicine, Morrisville, NC, [6]Foundation Medicine, Inc., Morrisville, NC, [7]Foundation Medicine, Inc, Morrisville, NC, [8]Foundation Medicine, Cambridge, MA, [9]Cambridge, MA

Disclosures:
Jeffrey Ross: *Employee*, Foundation Medicine
Jon Chung: *Employee*, Foundation Medicine
Jo-Anne Vergilio: *Employee*, Foundation Medicine
Shakti Ramkissoon: *Employee*, Foundation Medicine
James Suh: *Employee*, Foundation Medicine
Eric Severson: *Employee*, Foundation Medicine
Ryan Hartmaier: *Employee*, Foundation Medicine
Alexa Schrock: *Employee*, Foundation Medicine
Siraj Ali: *Employee*, Foundation Medicine
Philip Stephens: *Employee*, Foundation Medicine
Laurie Gay: *Employee*, Foundation Medicine

Background: Classic lobular (CILC) and pleomorphic lobular (PILC) breast care classified by loss of E-cadherin expression, but feature significant histologic differences. We queried whether comprehensive genomic profiling (CGP) of relapsed metastatic mCILC and mPILC, would also harbor contrasting genomic alterations (GA) that would differentiate the 2 subtypes and influence therapy selection.

Design: From 10,784 FFPE clinically advanced metastatic BC samples, 550 (5%) mCILC including 428 *CDH1* mutated and 122 *CDH1* WT tumors and 26 *CDH1* mutated mPLIC were sequenced using hybridization-captured, adaptor ligation-based libraries to a mean coverage depth >600X for up to 315 cancer-related genes. Tumor mutational burden (TMB) was determined on 1.1 Mbp of sequenced DNA and microsatellite instability (MSI) was determined on 114 loci.

Results: : All 3 subtypes had a similar age (Table). Slide based ER+ positivity and HER2+ negativity was significantly more frequent in both mCILC groups than in mPILC (P<0.0001). The frequency of base substitutions in *ESR1* was significantly higher in both mCILC groups, and this difference was also significantly higher in metastasis biopsies exposed to hormonal therapy than in pre-treatment primary tumors (P<0.0001). *ERBB2* (*HER2*) GA (amp + non-amp) detected by CGP were higher in mPILC than in both mCILC groups in both pre-and post-treatment samples (P<0.0001 for all comparisons). The *ERBB2* GA frequency was nearly twice as high after hormonal therapy in all 3 groups. *ESR1* and *ERBB2* GA were mutually exclusive overall and especially in the mCILC groups. *PIK3CA* GA were the most frequent GA in both all 3 groups. *TP53* GA were significantly more frequent in mPILC than in either mCILC group. At 19%, the frequency of TMB ≥ 15 mutations/MB in mPILC was more than twice as frequent than in *CDH1* mut mCILC (P=0.046). All (100%) mCILC and mPILC were negative for mis-match repair deficiency or MSI high status. mCILC and mPILC patient responses to precision therapies will be presented.

	CDH1 Mutated mCILC (428)	**CDH1** Wild Type mCILC (122)	**CDH1** Mutated mPILC (26)
Median Age	63	62	63
*ER+	98%	86%	74%
*HER2 IHC/ FISH+	3%	2%	22%
TNBC	2%	16%	6%
ESR1 GA Primary Pre-Rx	6%	5%	0%
ESR1 GA Metastatic Post-Rx	17%	19%	0%
ERBB2 GA Primary Pre-Rx	7%	8%	18%
ERBB2 GA Metastatic Post-Rx	12%	8%	34%
Other Significant GA	PIK3CA (55%), CCND1 (21%), TP53 (17%), ARID1A, AKT3, MDM4, PTEN (all 11%)	PIK3CA (36%) TP53 (13%) PTEN (12%) CCND1 (12%)	PIK3CA (58%), TP53 (30%), AKT1 22%), FGFR4, CCND1, PTEN (all 17%)
TMB median (mut/Mb)	2.7	3.6	3.6
TMB ≥ 15 mut/Mb	8%	7%	19%

*when clinical status available

Conclusions: CGP of *CDH1* mut and WT mCILC and mPILC reveals significant differences in the panorama of GA both in pre-treatment primary and metastatic disease lesions especially in therapy-impacting GA in *ESR1* and *ERBB2*. mCILC is more often driven by *ESR1* GA and mPILC by *ERBB2* GA. Although both mCILC and mPILC feature subsets of tumors with high TMB, this is more frequent for mPILC likely indicating different potentials for immunotherapies to benefit these patients.

287 Differential Expression of Nicotinamide N-methyltransferase (NNMT) in Stroma of Invasive Breast Cancer

Madhuchhanda Roy[1], *Karla Esbona*[2], *Ernst Lengyel*[3], *Mark Eckert*[3], *Stephanie M McGregor*[4]. [1]Madison, WI, [2]University of Wisconsin, [3]University of Chicago, [4]University of Wisconsin, Madison, WI

Background: Nicotinamide N-methyltransferase (NNMT) is expressed in multiple cancers and in some cases is associated with a poor prognosis in comparison to cases with lower expression. It has also been implicated as a potential mediator of resistance to chemotherapy. Though down-regulation of NNMT in breast cancer cells lines has been shown to induce apoptosis, its expression in primary human breast tumors has not been evaluated. The goal of this work was to characterize the expression of NNMT in carcinoma of the breast and to determine if it holds prognostic implications.

Design: 372 of formalin-fixed, paraffin-embedded breast cancer were used to construct a tissue microarray in triplicate cores. Following exclusion of cases without represented invasive carcinoma, 230 cases of invasive ductal carcinoma (IDC, including 6 tubular and 4 mucinous), 18 invasive lobular carcinoma (ILC), and 15 mixed carcinomas with a lobular component (ILC-mixed) were subjected to immunohistochemistry for NNMT. Stromal expression was scored semi-quantitatively as negative, <10%; focal, 10-50%; or diffuse, >50%. Tumor cells were scored as positive or negative according to the presence or absence of expression. In addition, 15 control cases from breast reduction served as controls. Chi square analysis was used for statistical comparisons.

Results: No NNMT expression was identified in the stroma of any benign breast parenchyma, though it was frequently seen in myoepithelial cells. NNMT expression was identified in tumor cells from 10% of IDC but in 0% of ILC; ILC-mixed demonstrated NNMT expression in 7% of cases. In contrast to the low incidence of expression within the tumor cells per se, NNMT expression was common in the stromal component, and much more so in IDC than in ILC or ILC-mixed (68%, 28%, and 47%, respectively; p=0.036 for IDC vs. ILC). Stromal expression was scored as diffuse in over 70% of cases. Of note, 100% of cases of tubular carcinoma demonstrated stromal NNMT expression, in contrast to 0% of the mucinous cases. No appreciable difference in other clinicopathologic parameters was identified, including grade, tumor size, nodal status, and recurrence.

Conclusions: In summary, NNMT is differentially expressed in the stroma of invasive breast carcinoma, specifically between ductal and lobular histotypes and may have a role in regulation of the tumor microenvironment. Further study is necessary to determine if NNMT expression holds prognostic significance.

288 Predictive Value of Tumor-Infiltrating Lymphocytes to Pathological Complete Response in Neoadjuvant Treated Triple-Negative Breast Cancers

Miao Ruan[1], *Tian Tian*[1], *Jia Rao*[2], *Xiaoli Xu*[3], *Bao-Hua Yu*[4], *Wentao Yang*[5], *Ruohong Shui*[6]. [1]Fudan University Shanghai Cancer Center, Shanghai, [2]Fudan University Shanghai Cancer Center, Shanghai, [3]Fudan University Shanghai Cancer Center, Fudan University Shanghai Medical College, Shanghai, [4]Fudan University Shanghai Cancer Center, Shanghai, [5]Fudan University Shanghai Cancer Center, Shanghai, China, Shanghai Medical College, Fudan University, Shanghai, China, [6]Fudan University Shanghai Cancer Center

Background: Tumor-infiltrating lymphocytes (TILs) may have predictive value to pathological complete response (pCR) in neoadjuvant treated triple-negative breast cancers (TNBCs), However, lacking of standardized methodologies in TILs evaluation has hindered its application in clinical practice.

Design: To evaluate the predictive value of tumor-infiltrating lymphocytes (TILs) scored by methods recommended by International TILs Working Group 2014, we performed a retrospective study of TILs in 166 core needle biopsy specimens of primary invasive triple-negative breast cancers (TNBCs) with neoadjuvant chemotherapy (NAC) in a Chinese population. Intratumoral TILs (iTILs) and stromal TILs (sTILs) were scored respectively. The correlation between TILs and neoadjuvant chemotherapy response was analyzed, and we also explored optimal threshold of TILs levels to predict pCR.

Results: Both sTILs (P = 0.0001) and iTILs (P = 0.001) were associated with pCR in univariate logistic regression analysis. Multivariate logistic regression analysis indicated that both sTILs (P = 0.006) and iTILs (P = 0.04) were independent predictors for pCR. Receiver operating characteristics (ROC) curve analysis was used to identify the optimal thresholds of TILs. TNBCs with more than 20% sTILs (P = 0.001) or with more than 10% iTILs (P = 0.003) were associated with higher pCR rates in univariate analysis. Multivariate analysis showed that a 20% threshold of sTILs (P = 0.005) was an independent predictive factor for pCR.

Conclusions: In conclusion, our study indicated that TILs scored by recommendations of International TILs Working Group 2014 in pre-NAC core needle biopsy specimens was significantly correlated with pCR in TNBCs, higher TILs scores predicting higher pCR rate. Both sTILs and iTILs were independent predictors for pCR in TNBCs. A 20% threshold for sTILs may be feasible to predict pCR to NAC in TNBCs.

289 Distribution of PAM50 Intrinsic Subtypes in Primary Invasive Breast Cancer and Morphologically Normal Tumor-Adjacent Epithelium: Report from the Nurses' Health Study

Christopher Rubadue[1], *Gabrielle Baker*[2], *Jun Wang*[3], *Xuehong Zhang*[4], *Kevin Kensler*[5], *Andreea Stancu*[6], *Michael Pyle*[6], *Laura Collins*[2], *Heather Eliassen*[4], *Rulla Tamimi*[7], *Susan Hankinson*[8], *Yujing Jan Heng*[2]. [1]Beth Israel Deaconess Medical Center, Boston, MA, [2]Beth Israel Deaconess Medical Center, Boston, MA, [3]University of Southern California, Los Angeles, CA, [4]Brigham and Women's Hospital, [5]Harvard T.H. Chan School of Public Health, Boston, MA, [6]Beth Israel Deaconess Medical Center, [7]Harvard T.H. Chan School of Public Health, [8]University of Massachusetts

Background: PAM50 is a 50-gene assay that classifies breast cancer (BrCa) into five molecular subtypes: Luminal (Lum) A and B, HER2-enriched (HER2-E), Basal-like (BL), and Normal-like (NL). Recent studies suggest that morphologically normal tumor-adjacent epithelium (N-ADJ) may provide additional insights into BrCa, and that PAM50 subtypes are reflected in N-ADJ. This study reports the creation of one of the largest BrCa and N-ADJ gene expression datasets. Specifically, we report the distribution and pairings of PAM50 in tumor and N-ADJ in the Nurses' Health Studies (NHS) and NHSII and provide comparison of PAM50 tumor subtypes with surrogate subtyping via immunohistochemistry (IHC).

Design: Tissue blocks from NHS (n=575) and NHSII (n=345) participants with primary invasive BrCa were collected. Regions of invasive BrCa and N-ADJ were annotated and cored, RNA was extracted, and microarrays performed (n=845 tumors, n=674 N-ADJ, n=599 pairs). PAM50 subtypes were computed (Parker et al, 2009). IHC surrogate molecular subtyping was per that previously reported (Sisti et al, 2015).

Results: The distribution of tumor PAM50 subtypes is shown in Table 1. When stratified by estrogen receptor (ER) status, 74% of ER+ were Lum A/B and 52% of ER- tumors were BL. Among HER2-E tumors, 43% were HER2+ via IHC. Most N-ADJ tissues (52%) were classified as NL. The most common pairing was Lum A tumor with NL N-ADJ (Table 2). There was 54% concordance between PAM50 and surrogate IHC subtypes (kappa=0.3); concordance improved to 80% (kappa=0.5) when Lum tumors were combined.

Table 1.

PAM50 Subtype	Nurses' Health Study n (%)	Nurses' Health Study II n (%)	Total n
Luminal A	233 (43.4)	150 (48.7)	383
Luminal B	87 (16.2)	56 (18.2)	143
HER2-Enriched	83 (15.5)	42 (13.6)	125
Basal-Like	86 (16.0)	37 (12.0)	123
Normal-Like	48 (8.9)	23(7.5)	71

Table 2.

	Paired Normal-Adjacent				
Tumor Subtype	Luminal A	Luminal B	HER2-Enriched	Basal-Like	Normal-Like
Luminal A	76	2	14	33	160
Luminal B	15	9	7	8	59
HER2-Enriched	4	4	28	20	24
Basal-Like	11	0	2	39	37
Normal-Like	6	0	2	6	33

Conclusions: We have created the largest publicly available paired BrCa and N-ADJ gene expression database. The tumor PAM50 distribution agrees with published cohorts; and the majority of N-ADJ was subtyped as NL. This rich dataset, together with the well-characterized cohorts of the NHS/NHSII, will be a valuable resource for future molecular pathology epidemiology studies.

290 Comparison of Pre-Processing Methods to Compute PAM50 Intrinsic Subtype in Breast Cancer and Histologically Normal Tumor-Adjacent Regions

Christopher Rubadue[1], *Gabrielle Baker*[2], *Venkat Sankar*[3], *Joel Parker*[4], *Katherine Hoadley*[4], *Laura Collins*[2], *Heather Eliassen*[5], *Rulla Tamimi*[6], *Susan Hankinson*[7], *Yujing Jan Heng*[2]. [1]Beth Israel Deaconess Medical Center, Boston, MA, [2]Beth Israel Deaconess Medical Center, Boston, MA, [3]Beth Israel Deaconess Medical Center, [4]University of North Carolina, [5]Brigham and Women's Hospital, [6]Harvard T.H. Chan School of Public Health, [7]University of Massachusetts

Background: Prediction Analysis of Microarray 50-gene signature (PAM50) is a 50-gene assay that classifies breast cancer into five molecular subtypes: Luminal A, Luminal B, HER2-enriched, Basal-like, and Normal-like. The original dataset used to derive the PAM50 algorithm was composed of an equal distribution of estrogen receptor (ER) positive (+) and ER negative (-) tumors (Parker, 2009). However, this distribution does not accurately reflect the distribution of ER status in breast cancer in the general population (~80% ER+/20% ER-; Harvey, 1999). The accuracy of PAM50 classification is affected when the ER distribution of the new research cohort does not match the original dataset (Parker, 2009). To address that challenge, two pre-processing methods for computing PAM50 subtypes were subsequently established: the modified median gene centering method (MMGC; TCGA, 2012) and the subgroup-specific gene centering method (SSGC; Zhao, 2015). Although PAM50 was not developed for this purpose, investigators have begun to evaluate gene expression in histologically normal tumor-adjacent epithelium (N-ADJ) via PAM50. The purpose of this study was to compare PAM50 molecular subtypes computed by these two pre-processing methods in breast cancer and N-ADJ.

Design: Formalin-fixed paraffin-embedded tissue blocks (n=920) of primary invasive breast cancer were acquired from the Nurses' Health Study (NHS) and NHSII. H&E-stained slides were digitalized and annotated for breast cancer and N-ADJ. The designated regions were cored, RNA was extracted, and expression microarrays were created (n=845 tumors, n=674 N-ADJ). PAM50 subtypes were computed by the MMGC and SSGC methods for both tumor and N-ADJ. Concordance between the two methods was then evaluated for both regions.

Results: Between the two pre-processing methods, there was a concordance of 86% for the tumor tissue and 82% for the N-ADJ. The kappa values were both 0.8, demonstrating high agreement between the two pre-processing methods.

Conclusions: Research-based PAM50 subtyping by both pre-processing methods has high agreement and practical utility. Either method may be employed to classify breast tissue samples.

291 Breast Cancer HER2 (ERBB2) Status by Next-Generation Sequencing Compared to Traditional Methods

Meaghan K Russell[1], *Angeliki Pantazi*[2], *Stephen Lyle*[3], *Julie Y Tse*[4]
[1]KEW, Inc., [2]KEW, Inc., Lethbridge, AB, [3]Univ. of Massachusetts, Worcester, MA, [4]Tufts Medical Center, KEW Inc., Boston, MA

Disclosures:
Meaghan Russell: *Employee*, KEW, Inc.
Angeliki Pantazi: *Employee*, KEW, Inc.
Stephen Lyle: *Employee*, KEW, Inc.
Julie Tse: *Consultant*, KEW, Inc.

Background: Breast cancers with HER2 (ERBB2) amplification may be treated with FDA-approved anti-HER2 antibody therapies and small molecule ERBB2/EGFR inhibitors. Approximately 20% of breast cancers are HER2-amplified. Studies have also demonstrated that 3% of breast cancers have activating HER2 mutations, which confer response to small molecule ERBB2/EGFR inhibitors [PMID: 23220880] in tumors that are usually otherwise HER2 non-amplified [PMID: 28762010]. The current gold standard for determination of HER2 amplification status is immunohistochemistry (IHC) and/or in situ hybridization (ISH). Next generation sequencing (NGS) for alterations in cancer-related genes is increasingly used in solid tumors and can identify both copy number gains as a marker for gene amplification and activating mutations from a single, small input sample. We aim to investigate the ability of NGS to 1) determine HER2 status compared to traditional methods and 2) to detect activating mutations in HER2.

Design: Breast cancer cases were sequenced using CANCERPLEX®, a NGS platform of 435 cancer-related genes. Alterations detected in HER2 included non-synonymous single nucleotide polymorphisms, insertions and deletions, and copy number variation (CNV). HER2 amplification status was determined by CNV equal to or greater than 2.5-fold as the threshold for HER2 amplification, and compared to HER2 status by IHC and/or ISH.

Results: 150 cases of breast cancer from female patients were included in the study. By traditional IHC and/or ISH testing, 17.3% were previously determined to be HER2 amplified and 82.7% cases HER2 non-amplified. There was a >98% concordance between CANCERPLEX and IHC/ISH results in HER2 non-amplified tumors and an overall 91.3% concordance of HER2 amplification status. NGS also detected activating HER2 mutations in 3.3% of all cases, including 3.2% of HER2 non-amplified breast cancers.

Conclusions: Our study demonstrates strong concordance of HER2 status by NGS compared to traditional methods of IHC and/or ISH. Reasons for non-concordance included compromised DNA quality or quantity (such as sample decalcification) and low level HER2 amplification by ISH (less than 2.4 HER2/CEP17). Using NGS, we also detected activating HER2 mutations in 4% of cases, and thus, identified additional cases with potential to benefit from small molecule ERBB2/EGFR inhibitors. Overall, CANCERPLEX NGS offers comprehensive testing solution for breast cancer that is cost-effective and optimizes use of small biopsy specimens.

292 Lobular Carcinomas In Situ Display Intra-Lesion Genetic Heterogeneity and Clonal Evolution in the Progression to Invasive Disease

Michail Schizas[1], *Ju Youn Lee*[1], *Pier Selenica*[1], *Felipe Geyer*[2], *Salvatore Piscuoglio*[3], *Rita A Sakr*[1], *Charlotte K Ng*[3], *Raymond S Lim*[1], *Jose V Carniello*[4], *Russell Towers*[4], *Luciano G Martelotto*[5], *Dilip Giri*[6], *Anastasios Papanastasiou*[7], *Agnes Viale*[4], *David Solit*[4], *Britta Weigelt*[6], *Jorge Reis-Filho*[8], *Tari King*[9]. [1]MSKCC, [2]Memorial Sloan-Kettering Cancer Center, New York, NY, [3]University of Basel, [4]Memorial Sloan Kettering Cancer Center, [5]Memorial Sloan Kettering Cancer Center, New York, NY, [6]New York, NY, [7]Patras General Hospital, Patras Achaea, [8]Memorial Sloan-Kettering CC, New York, NY, [9]Brigham and Women's Hospital

Background: Lobular carcinoma *in situ* (LCIS) is considered both a risk indicator and non-obligate precursor to breast cancer yet the molecular underpinnings of this behavior remain unclear and there has been a movement away from staging LCIS as pTis. Here we sought to define the genomic landscape of LCIS, and to ascertain their clonal relatedness with synchronous more advanced lesions.

Design: 15 cases composed of multifocal and/or bilateral LCIS and synchronously diagnosed invasive lobular carcinoma (ILC), ductal carcinoma *in situ* (DCIS) and/or invasive ductal carcinoma (IDC) were subjected to central review and microdissection of each component separately. DNA samples (n=52) of LCIS (n=30), DCIS (n=7), ILC (n=10) and IDC (n=5) were subjected to whole-exome sequencing (WES). Somatic genetic alterations and mutational signatures were defined using validated bioinformatics algorithms. Clonal decomposition analysis was performed using a Bayesian clustering model (PyClone).

Results: WES analysis of LCIS revealed recurrent genetic alterations affecting genes found to be significantly mutated in ILCs and luminal A breast cancers from TCGA, such as *CDH1*, *PIK3CA* and *FOXA1*. 26 of 30 (87%) LCIS harbored *CDH1* pathogenic mutations, of which 24 were coupled with loss of heterozygosity of the wild-type allele. Fourteen LCIS were clonally related to DCIS (n=9) or ILC (n=5), whereas no LCIS was clonally related to IDC, suggesting that a direct progression from LCIS to IDC is a rare event. In 2 cases, a minor LCIS subclone was the likely substrate for the development of the DCIS and ILC, suggesting clonal selection, whereas in other cases parallel progression was observed. Intra-lesion genetic heterogeneity was higher among LCIS clonally related to DCIS or ILC (n=14) than in LCIS not clonally related to DCIS or ILC (p-value=0.01, t-test). Examples of changes in the mutational signatures in the progression from LCIS to DCIS or ILC were observed, in which the trunk mutations displayed the aging signature, whereas the clone that gave rise to the DCIS or ILC displayed the APOBEC-related mutational signature.

Conclusions: Our findings confirm that LCIS is a neoplastic lesion and often constitutes a non-obligate precursor of breast cancer, driven by *CDH1* inactivation. Our results also emphasize the importance of taking into account intra-lesion genetic heterogeneity in studies aiming to develop biomarkers of progression from LCIS to more advanced lesions.

293 Microglandular Adenosis with Somatic TP53 Mutation is a Clonally-Advanced Lesion with a Molecular Signature Significantly Overlapping with That of Its Corresponding Metaplastic Carcinoma

Christopher J Schwartz[1], *Esther Yoon*[2], *Igor Dolgalev*[3], *Eleazar Vega-Saenz de Miera*[3], *Adriana Heguy*[3], *Iman Osman*[2], *Farbod Darvishian*[4]. [1]New York University Medical Center, New York , NY, [2]New York University Medical Center, New York, NY, [3]New York University Medical Center, [4]New York University Medical Center, New York, NY

Background: Microglandular adenosis (MGA) is a rare borderline lesion of the breast characterized by an infiltrative collection of small glands that characteristically lack a myoepithelial cell layer. MGA is associated with invasive carcinoma in 20-30% of cases, and has been proposed as a non-obligate precursor to basal-like breast cancers. Somatic TP53 mutation of MGA and its associated carcinoma has been previously reported. We identified a case of triple negative metaplastic carcinoma with mesenchymal differentiation with morphologic and immunohistochemical evidence of progression from MGA to atypical MGA (AMGA), carcinoma in situ (CIS) and invasive carcinoma. We performed laser microdissection and whole exome sequencing of each four components (MGA, AMGA, CIS and cancer) with a matched benign sample to characterize the mutational landscape of these foci.

Design: We selected a case of a metaplastic carcinoma with mesenchymal differentiation in juxtaposition to foci of MGA, AMGA and CIS. Immunohistochemically, all four foci were negative for estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 (her2) and positive for S-100. The distinct morphologic components and a matched control lymph node were separately microdissected from formalin-fixed paraffin embedded blocks. DNA extraction was performed and subjected to whole-exome sequencing on Illumina HiSeq platform. The results were demultiplexed and converted to FASTQ format using Illumina bcl2fastq software. Single-nucleotide and small indel somatic variants were called with MuTect2. Copy number profiles were calculated using Control-FREEC. Clonal populations were identified and quantified using PyClone.

Results: Sequencing data resulted in mean coverage of 96-134X of targeted exome regions. Our results found a recurrent stop-gain *R213** TP53 mutation in MGA, atypical MGA, CIS and metaplastic carcinoma. In addition, through variant allele frequency analysis, we identified two putative clonal clusters shared by all foci indicating a common molecular signature that is preserved in the morphologic spectrum of MGA-AMGA-CIS-metaplastic carcinoma.

Conclusions: MGA is a molecularly advanced lesion with somatic mutation of TP53. We postulate that TP53 is an early event in the progression of MGA through AMGA, CIS and its associated metaplastic carcinoma. We report significant genetic overlap between MGA and its associated cancer.

294 Concordance and Efficacy of Intraoperative Gross Examination of Margin Vs Final Microscopic Margin in Breast Conserving Surgery: One Year Experience in a Large Metropolitan Health Care System

Alpa Shah[1], *Kimberly Cole*[2], *Dhananjay A Chitale*[3]. [1]Henry Ford Hospital, Royal Oak, MI, [2]Iowa City, IA, [3]Henry Ford Hospital, Detroit, MI

Background: Controversy still exists regarding the optimal margin width in breast-conserving surgery (BCS) for breast cancer, but negative pathological margin status is an important component of optimal management. Margin reexcision is the most common indication for reoperation in patients undergoing BCS. Intraoperative margin evaluation (IOM) is generally an uncommon practice in BCS, but recently, our institutional surgeons required IOM status on most BCS specimens. Effective freezing and optimal sectioning for microscopic evaluation of fatty breast tissues is quite challenging due to technical limitations. Therefore, our aim was to assess concordance

and efficacy of intraoperative gross examination of margin (IOGM) status with final microscopic margin.

Design: Over 1 year period, all consecutive cases of BCS specimens where IOM status was requested by the surgeon were included in this study. For each case, a standard gross examination protocol was followed. Briefly, specimens were oriented, inked and serially sectioned at 3 mm thick slices. Gross observation of cut surfaces and a thorough palpation of each slice to identify firm tumor or biopsy cavity were carried out to assess closest margin distance. Margin status was reported with tumor / biopsy cavity distance to the closest margins as negative, close (< 1 mm) or positive. Positive and close margins prompted additional margin excision. Discordance with impact on clinical management was considered when IOGM was reported negative and microscopic final margin was either positive or close (<1 mm). Histological subtypes and other relevant clinicopathologic findings such as neoadjuvant status, primary vs recurrent tumor were recorded.

Results: 176 women contributed to this study. There were 172/176 BCS, 2/176 mastectomies, 2/176 re-excisions. Primary diagnoses for all cases are listed in Table 1. The concordance between gross and final microscopic margin status is shown in Table 2. 13/176 (7.4%) cases had margins that were called negative on IOGM and turned out positive or close on microscopic final margin status (4 DCIS, 6 IDC-NOS, 1 IDC with lobular features, 1 IDC status post neoadjuvant chemotherapy, 1 ILC).

Table 1

Primary diagnoses	Total	Percent
Invasive ductal carcinoma (IDC)-NOS	128	72.7%
Invasive lobular carcinoma (ILC)	19	10.8%
Metaplastic carcinoma	3	1.7%
Mucinous carcinoma	1	0.6%
Adenomyoepithelial carcinoma	1	0.6%
Ductal carcinoma in-situ (DCIS)	18	10.2%
Intracystic papillary carcinoma	2	1.2%
Benign diagnoses	4	2.3%
Grand Total	176	100%

Table 2

	Micro-Negative	Micro-Close (<1mm)	Micro-Positive	Grand Total
Gross-Negative	120	7	6	133
Gross-Close (<1mm)	6	19	2	27
Gross-Positive	5	4	7	16
Grand Total	131	30	15	176

Conclusions: There was 93% concordance between IOGM negative status and microscopic specimen margins in this cohort. IOGM dramatically improved intraoperative TAT and reduced reoperation rates. Our study highlights a practical, efficient and easily adaptable method of IOM status evaluation.

295 Genomic Profiling of Lobular Carcinoma in Situ (LCIS) Variants with Comparison to Classic LCIS and Invasive Lobular Carcinoma

Eliah Shamir[1], *Tianming Chu*[2], *Yunn-Yi Chen*[3], *Gregor Krings*[4]. [1]University of California, San Francisco, San Francisco, CA, [2]UCSF, [3]Univ. of California, San Francisco, San Francisco, CA, [4]UCSF, San Francisco, CA

Background: Whether pleomorphic (PLCIS) and florid (FLCIS) lobular carcinoma in situ variants (vLCIS) should be treated as high-grade precursors akin to ductal carcinoma in situ is controversial. The 2018 AJCC guidelines no longer classify PLCIS or FLCIS as pTis due to low prevalence and insufficient outcomes data. However, PLCIS and FLCIS have more aggressive biomarker profiles and increased copy number alterations (CNA) than classic LCIS (cLCIS) and are often associated with invasive lobular carcinoma (ILC). The genetics of vLCIS, including relationship to cLCIS and ILC, remain largely unknown. We used capture-based next generation sequencing (NGS) to profile paired cLCIS, vLCIS and ILC, as well as vLCIS not associated with ILC, to gain insight into their biology and help inform classification.

Design: DNA was extracted from 9 synchronous cLCIS, vLCIS (5 PLCIS and 4 FLCIS) and ILC, and 5 pure vLCIS (3 PLCIS and 2 FLCIS) without associated ILC. NGS was performed targeting exons of 480 cancer-related genes. Single nucleotide variants, small insertions/deletions and CNA were analyzed.

Results: Shared pathogenic mutations and CNA were identified between vLCIS and ILC in 9/9 cases and between cLCIS and vLCIS/ILC in 8/9 cases, with cLCIS clonally unrelated to paired vLCIS/ILC in 1 case. Recurrent mutated genes in vLCIS/ILC included *CDH1* (13/14), *PIK3CA* (8/14), *ERBB2* (6/14), *ERBB3* (3/14) and *TP53* (2/14); 3/14 had *CCND1* amplification (amp). *ERBB2* and/or *ERBB3* mutations were present in 50% vLCIS (4/8 PLCIS, 3/6 FLCIS), including 3/9 (33%) vLCIS associated with ILC and 4/5 (80%) pure vLCIS. *ERBB2* and *ERBB3* mutations were present in cLCIS of 2 cases. Progression to vLCIS was associated with *TP53* and *ERBB2*/*ERBB3* mutations (1 case each), *CCND1* amp (1 case) and more CNA (4/8 cases). Progression to ILC was associated with *NF1*, *ASXL2* and *FOXA1* mutations (1 case each) and more CNA (6/9 cases). Mean CNA were similar between pure vLCIS (4, range 2-7) and vLCIS associated with ILC (6.6, range 1-17;p=.378).

	vLCIS	vLCIS ER/PR/HER2	Invasive carcinoma	Subtype	SBR grade	ILC ER/PR/HER2	**ERBB2** mutation cLCIS/vLCIS/ILC	**ERBB3** mutation cLCIS/vLCIS/ILC
P1	PLCIS	+ / + / -	ILC	classic	2	+ / + / -	- / - / -	- / - / -
P2	PLCIS	- / - / -	PILC	pleomorphic	3	- / - / -	NA / - / -	- / - / -
P3	PLCIS	+ / NA / -	PILC	pleomorphic, alveolar	2	+ / - / -	+ / + / +	- / - / -
P4	PLCIS	+ / - / -	PILC	pleomorphic	2	- / - / -	- / + / +	- / + / +
P5	PLCIS	+ / - / -	PILC	pleomorphic	2	+ / - / -	- / - / -	- / - / -
F1	FLCIS	+ / + / -	PILC	pleomorphic, solid	3	+ / + / -	- / - / -	- / - / -
F2	FLCIS	+ / + / -	ILC	classic, alveolar	2	+ / + / -	NA / - / -	- / - / -
F3	FLCIS	+ / + / -	ILC	classic	2	+ / - / -	+ / + / +	- / - / -
F4	FLCIS	NA / NA / -	ILC	classic, alveolar	2	+ / + / -	- / - / -	- / - / -

Pure vLCIS	vLCIS	vLCIS ER/PR/HER2	**ERBB2** mutation	**ERBB3** mutation
pP1	PLCIS	+ / - / -	+	-
pP2	apocrine PLCIS	- / - / -	-	-
pP3	apocrine PLCIS	- / - / -	+	-
pF1	FLCIS	+ / + / -	+	+
pF2	FLCIS	+ / - / -	-	+

Conclusions: Synchronous cLCIS, vLCIS and ILC are clonally related with similar mutation profiles, but ILC have more CNA and pathogenic mutations compared to LCIS. *ERBB2* and *ERBB3* pathogenic mutations are enriched in HER2 negative vLCIS and associated ILC but are also present in associated cLCIS. The results provide evidence that vLCIS progresses from cLCIS and is a direct precursor to ILC. Frequent *ERBB2* mutations in these cancers are consistent with more aggressive behavior and may have treatment implications.

296 Triple Positive Breast Cancers: A Histopathologic and Clinicopathologic Review

Elisheva Shanes[1], *William Watkin*[2], *Megan E Sullivan*. [1]NorthShore University HealthSystem, Evanston, IL, [2]NorthShore University Health System, Evanston, IL

Background: While "triple negative" breast carcinomas are recognized as a distinct subgroup with clinical significance, there is a paucity of literature on the subgroup of invasive breast carcinoma with positive IHC staining for both hormone receptors (ER and PR) as well as HER2 ("triple positive," TP). HER2 positivity is identified in 15-20% of invasive carcinomas of the breast and is associated with decreased disease-free and overall survival. Approximately 50% of HER2 positive tumors are also ER/PR positive and clinical outcome studies suggest that these patients may follow a more aggressive course. This study examines the histologic characteristics of TP tumors and compares patients with TP to ER/PR negative-HER2 positive (HER2) tumors.

Design: Patients with ER/PR/HER2 IHC results on invasive breast carcinoma between 1/1/2014 and 6/1/2017 were identified via the electronic pathology record. A TP cohort and a HER2 cohort were identified. Clinical information, including age, staging, treatment and follow-up, was extracted from the patients' medical records. Slides from the TP tumors were reviewed and graded using the Nottingham system

Results: 44 TP patients were identified and slides were available for review in 40 cases. 39 were invasive ductal carcinomas (3 with micropapillary and 1 with mucinous features). There was one invasive lobular carcinoma. The average tumor grade was 2.3 (architectural score 2.7, nuclear score 2.5 and mitotic activity score 1.9). 15 (38%) had tumor infiltrating lymphocytes (TILs).

Clinical features of the TP cohort were compared to 49 HER2 patients. The average age of TP patients was 54 (25-85) and the average age of HER2 patients was 60 (36-82). 18 (41%) TP patients vs. 10 (20%) HER2 patients were under age 50 at diagnosis. 17 TP and 18 HER2 patients underwent primary surgical excision. 12% overall (TP and HER2) were stage 4 at diagnosis.

Overall, 39% (36/93 total patients) received neoadjuvant chemotherapy (NAC). There were 21 (43%) HER2 NAC patients and 20 (95%) achieved pathologic complete response (pCR). Of 15 (34%) TP NAC patients, 6 (40%) achieved pCR. Of the TP NAC patients with a pCR, 67% (4/6) had associated TILs.

Conclusions: Patients with TP breast cancers are younger than patients with HER2 cancers. In spite of the availability of HER2 receptor blockers for both cohorts, TP patients are significantly less likely to achieve pCR with NAC (p<0.01). While they may represent a distinct clinical group, TP tumors do not have unique histologic features.

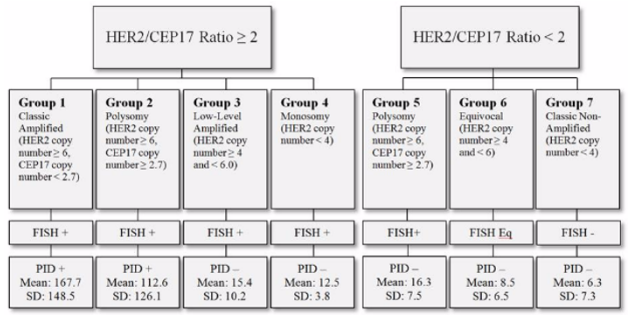
297 Quantitative Measurement of Human Epidermal Growth Factor Receptor-2 (HER2) Protein Expression in 'Classical' and 'Non-Classical' FISH Categories: a Comparative Study

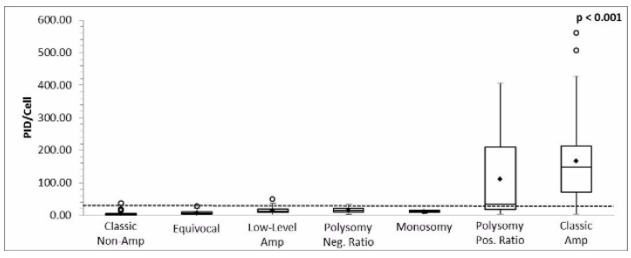
Jian Shen[1], *Brandon Buscaglia*[2], *Hideki Goda*[3], *Bradley M. Turner*[4], *Hisatake Okada*[5], *Loralee Mcmahon*[2], *Jill Henry*[2], *Yasushi Nakano*[6], *David Hicks*[7]. [1]University of Rochester Medical Center, Rochester, NY, [2]University of Rochester Medical Center, [3]Konica Minolta INC., Hino, Tokyo, [4]University of Rochester Medical Center, Rochester, NY, [5]Konica Minolta INC., Hino-city, Tokyo, [6]Konica Minolta,Inc., Tokyo, [7]Rochester, NY

Background: Targeting HER2 protein overexpression in breast cancer has been shown to be an effective therapeutic modality. One methodology of assessing HER2-status is fluorescent in situ hybridization (FISH). FISH evaluates HER2 gene amplification, which is a surrogate for protein expression. FISH results are classified based on the HER2/CEP17 ratio and HER2 gene copy number. FISH relies on the assumption that the HER2 gene copy numbers accurately reflect the amount of protein that is translated in tumor cells. In the current study, we use a novel immunodetection methodology utilizing streptavidin coated Phosphor-integrated dot fluorescent nanoparticles (PID) to quantitatively measure HER2 protein expression in different FISH categories.

Design: 159 cases of invasive breast cancers, which had previously undergone HER2 FISH testing, were selected for this study. Cases were sorted and categorized into 'classical' (groups 1 and 7) and 'non-classical' (groups 2-6) FISH categories (Figure 1). PID testing was performed on all cases, and the PID HER2 protein expression was compared to HER2 FISH results by category.

Results: Both 'classical' FISH categories correlated, as would be expected, with HER2 protein expression (Figures 1 and 2). However, 'non-classical' FISH categories were found to have very low-levels of HER2 protein expression, except for polysomy ratio positive cases (group 2), which had similar protein expression to 'classical' FISH amplified cases (Figures 1 and 2) (P < 0.001).





Conclusions: Our results show that HER2 protein expression in four out of five 'non-classical' FISH categories (groups 3-6) were all comparable to the 'classical' non-amplified FISH category when measured by PID. This suggests that these 'non-classical' FISH categories may be less likely to respond to targeted HER2 therapy. Furthermore, HER2 protein expression in group 2 was comparable to the 'classical' amplified FISH category when measured by PID. This suggests that this 'non-classical' FISH category may be more likely to respond to targeted HER2 therapy. The findings of this study show that neither HER2/CEP17 ratio, nor HER2 gene copy number alone can accurately predict the HER2 protein expression in all cases. The correlations between PID HER2 protein expression and FISH categories suggests that quantification of HER2 protein with PID will add value in determining HER2 status for targeted HER2 therapy. Follow up studies with a larger patient cohort are warranted.

298 Unusual Expression of Immunohistochemical Markers PAX8 and CDX2 in Breast Cancer

Tiansheng Shen[1], *Jun Zhao*[2], *Min Zhao*[3], *Melissa W. Taggart*[4], *Preetha Ramalingam*[5], *Yun Gong*[6], *Yun Wu*[7], *Lei Huo*[8]. [1]UT MD Anderson Cancer Center, Houston, TX, [2]M D Anderson Cancer Center, Houston, TX, [3]MD Anderson Cancer Center, [4]UT MD Anderson Cancer Center, Houston, TX, [5]M. D. Anderson Cancer Center, Houston, TX, [6]UT-MD Anderson Cancer Center, Houston, TX, [7]Houston, TX, [8]UT MD Anderson Cancer Center

Background: Tissue-specific immunohistochemical markers are particularly helpful in characterizing tumor origin, an important step leading to appropriate clinical management. Knowledge of the sensitivity and specificity of such markers in tumor of each organ site is crucial for their accurate diagnostic interpretation. PAX8 has been used as a marker specific for gynecological tract, kidney and thyroid tumors. CDX2 is a marker of gastrointestinal carcinomas. Neither is considered as a breast tumor marker. However, we have encountered breast tumors that express either marker in our routine practice, some of which causing diagnostic confusion and extensive clinical workup. Our aim in this study is to investigate the expression of PAX8 and CDX2 in breast cancer.

Design: A Pubmed search was conducted using key words "PAX8" AND "breast" or "CDX2" AND "breast". Studies that included staining of either marker in breast cancer cases were selected for review. Pathologically and/or clinically confirmed primary or metastatic breast carcinomas that had PAX8 or CDX2 staining results reported between 2001 and 2017 were identified by searching our pathology file. Tissue microarrays (TMA) constructed from primary breast cancer resection specimens of patients diagnosed between 2006 and 2016 were stained for PAX8 or CDX2 by immunohistochemistry. Any nuclear staining was considered positive.

Results: The results for PAX8 and CDX2 expression in breast cancer are summarized in Table 1. A total of 247 tumors in our pathology file review had PAX8 staining results reported during diagnostic workup, including 108 (44%) primary and 139 (56%) metastatic breast cancers. Seven primary and 4 metastatic tumors were positive for PAX8, including 3 with rare scattered cells stained, 5 with focal and weak staining, and 3 with strong and diffuse staining. The 292 tumors with reported CDX2 staining results included 85 (29%) primary and 207 (71%) metastatic breast cancers. Four primary and 1 metastatic triple-negative tumors were positive for CDX2, including 3 with focal and weak staining, 1 with patchy and moderate staining, and 1 with strong and diffuse staining. One triple-negative tumor in the TMAs was positive for CDX2, with weak diffuse staining.

Table 1. Expression of PAX8 and CDX2 in breast cancer

		Literature review		Pathology file review		TMA staining	
Marker		PAX8	CDX2	PAX8	CDX2	PAX8	CDX2
Total case No.		844[a]	775[b]	247	292	355	155
Positive case No.	Total (%)	0 (0%)	2 (0.3%)	11 (4.5%)	5 (1.7%)	0 (0%)	1 (0.6%)
	ER+/HER2-	n/a	n/a	3	0	0/206	n/a
	HER2+	n/a	n/a	1	0	0/45	0/45
	Triple-negative	n/a	n/a	7	5	0/104	1/110

[a]From 13 studies; [b]from 18 studies.

Conclusions: PAX8 and CDX2 can be infrequently expressed in breast cancer with various degrees of staining including strong and diffuse staining in rare cases, which can potentially result in erroneous diagnosis with regard to tissue origin.

299 Bacteria-Associated Granulomatous Mastitis: A Ten-Year Retrospective Review.

Olubunmi Shoyele[1], *Debasmita Das*[2], *Ramapriya Vidhun*[2], *Jessica Dodge*[3], *Zandra Cheng*[2], *Steven Sieber*[2]. [1]Western Connecticut Health Network, Danbury Hospital, Danbury, CT, [2]Western Connecticut Health Network, Danbury Hospital, [3]Western Connecticut Health Network, Danbury Hospital, Danbury, CT

Background: Granulomatous mastitis is a benign inflammatory disease of the breast with a broad etiology which could be bacterial, fungal, mycobacterial or autoimmune. Prior studies have identified the role of gram-positive bacilli such as *Corynebacterium* species in granulomatous mastitis. Studies have reported distinct histologic features which include granulomatous and neutrophilic inflammation surrounding clear cystic spaces. This has been termed "cystic

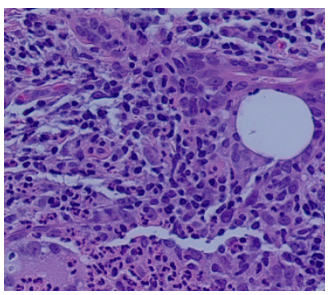
neutrophilic granulomatous pattern" of inflammation. Rare gram-positive bacilli are sometimes identified within these cystic spaces. Patients with cystic neutrophilic granulomatous mastitis (CNGM) are more likely to be Hispanic, younger or born outside of the United States. *Corynebacterium* species are difficult organisms to grow in the laboratory without special media. Hence, etiology of mastitis may be misdiagnosed as idiopathic.

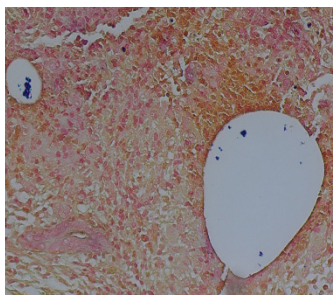
Design: A search of the "CERNER Millennium" database was conducted using the free text search for the words "breast", "granulomatous", and "mastitis". Histology slides were reviewed for pattern of inflammation. Retrospective gram stains were performed on eight cases. Bacterial, mycobacterial, fungal culture results and treatment data were documented. The pattern of inflammation was assessed to correlate the cystic neutrophilic granulomatous pattern of *Corynebacterium* -associated mastitis with gram stain and culture results.

Results: A total of fourteen cases were diagnosed as "granulomatous mastitis" over a ten-year period. All acid-fast bacilli and fungus stains performed were negative for mycobacterial and fungal organisms. Eight specimens from seven patients (62%) had the cystic neutrophilic granulomatous histologic pattern of inflammation (Table 1). Only two of the seven patients had a diagnosis of CNGM. Histology slides showed non-necrotizing granulomatous inflammation with neutrophilic microabscesses surrounding clear cystic spaces (Figure 1). Gram-positive bacilli were identified in four cases (Figure 2). *Corynebacterium amycolatum* was isolated in one patient whose specimen was sent to a reference laboratory. Five of the seven patients were Hispanic (71%). Treatment varied from none to a combination of antibiotics, immunosuppressant therapy and surgery.

Table 1. Clinicopathologic characteristics of patients with cystic neutrophilic granulomatous pattern of inflammation

Gram Stain	Bacterial Culture	Procedure	Ethnicity	Age	Treatment
Positive	Negative	Core needle biopsy	Hispanic	35	No treatment
Positive	Corynebacterium amycolatum isolated	Core needle biopsy	Hispanic	41	Antibiotics, steroids, methotrexate, superficial incision and drainage
Negative	Propionibacterium acnes isolated	Excisional biopsy	Non-Hispanic	31	Surgery
Negative	Normal cutaneous flora	Core needle biopsy	Hispanic	53	No treatment
Positive	Negative	Core needle biopsy	Hispanic	42	Antibiotics, steroids
Positive	Not performed	Core needle biopsy	Hispanic	46	Antibiotics, steroids, methotrexate
Negative	Not performed	Core needle biopsy	Non-hispanic	55	No treatment
Paraffin block unavailable	Not performed	Core needle biopsy	Non-Hispanic	31	Surgery





Conclusions: Although our sample size is small, CNGM is a distinct histopathologic entity which may be underdiagnosed by pathologists. Recognition of this pattern of mastitis is important so that patients may receive appropriate therapy.

300 Promitotic and Cyclin-Dependent Kinase Inhibitor Proteins Show Significant Correlation with Distant Metastasis in Breast Cancer Patients

Jaime Singh[1], *Nitai Mukhopadhyay*[2], *Michael Idowu*[3]. [1]Virginia Commonwealth University Health Systems, Richmond, VA, [2]VCU Health Systems, [3]Virginia Commonwealth Univ Health System, Richmond, VA

Background: The interplay of promitotic proteins and CDK4/CDK6 inhibitors has a significant role in determining progression of tumor cells from G1 to S phase of the cell cycle. Overexpression of promitotic G1/S proteins like CCND1 (Cyclin D1) and formation of the CDK4/CDK6-Cyclin D1 complex has been implicated in eventual resistance of estrogen receptor (ER) positive breast cancer to anti-estrogen treatment. CDK inhibitors (CDKI) like CDKN2A (p16), CDKN1A (p21) and CDKN1B (p27) are believed to act to prevent the CDK4/6-Cyclin D1 complex, limiting progression of tumor cells from G1 to S phase. Conversely, inactivation of CDKI leads to unopposed CDK4/6-Cyclin D1 complex formation and progression from G1 to S phase of the cell cycle. The aim of this study was to evaluate the correlation of promitotic proteins like Cyclin D1 and CDKI proteins like p16, p21 and p27 with occurrence of distant metastasis in breast cancer patients.

Design: Breast cancer cases with adequate clinical-pathologic follow-up information were retrieved between 2005 and 2013. Follow up information was collected via patient electronic medical records and the Cancer Registry. Tissue microarrays were constructed (1.0 mm cores, 3 cores per case). Immunohistochemistry for Cyclin D1, p16, p21/WAF1 and p27/KiP1 was performed. The Allred scoring system (sum of proportion and intensity) was used to evaluate nuclear reactivity. Cases with at least one scorable core were considered evaluable. Logistic regression with single covariate was used to calculate statistical significance; p-value <0.05 was considered significant.

Results: There were 193 evaluable cases. Overexpression of promitotic protein Cyclin D1 showed a significant direct association with likelihood of distance metastasis while overexpression of CDKI p16 showed a significant inverse association with distant metastasis (Table 1a). Expression of CDKI p27 was directly associated with distant metastasis similar to that of a promitotic protein. Overexpression of CDKI p21 showed no significant association with distant metastasis.

N=193	Analysis of maximum likelihood estimates	Odds ratio (95% confidence limits)	Correlation to Distant metastasis (p value)
CCND1 (Cyclin D1)	0.1762	1.193 (1.061-1.341)	0.0032
CDKN2A (p16)	-0.1544	0.0857 (0.774-0.948)	0.0029
CDKN1A (p21)	0.1801	1.197 (0.993-1.444)	0.0590
CDKN1B (p27)	0.1686	1.184 (1.040-1.347)	0.0104

Table 1a. Logistic regression of correlation between promitotic and antimitotic proteins and breast cancer distant metastasis

Conclusions: Overexpression of CDKI like p16 or promitotic proteins like Cyclin D1 may prove to be useful prognostic biomarkers for distant metastasis in breast cancer patients. Variable expression and correlation of CDKI like p27 and p21 may be due to sequestration of these proteins by CDK4-Cyclin D1 complex. Additional studies may be needed to further elucidate CDKI and promitotic protein significance in tumor cell metastasis.

301 Correlation of Cell Cycle Regulator Proteins With Distant Metastasis in Triple and Non-Triple Negative Breast Cancer

Jaime Singh[1], *Nitai Mukhopadhyay*[2], *Michael Idowu*[3]. [1]Virginia Commonwealth University Health Systems, Richmond, VA, [2]VCU Health Systems, [3]Virginia Commonwealth Univ Health System, Richmond, VA

Background: Overexpression of promitotic G1/S proteins and formation of the CDK4/CDK6-CCND1 (Cyclin D1) complex has been implicated in eventual resistance of estrogen receptor (ER) positive breast cancer to anti-estrogen treatment. CDK inhibitors (CDKI) like CDKN2A (p16), CDKN1A (p21) and CDKN1B (p27) act to prevent the formation of the CDK4/6-Cyclin D1 complex, thereby limiting progression of tumor cells from G1 to S phase. CDK4/6 inhibitor therapy with agents such as palbociclib is currently being used for advanced or recurrent ER positive breast cancer. Mutations/alterations in tumor suppressor gene TP53 (p53) activity occur in several different types of cancer and cancer syndromes. BCL2 is an anti-apoptotic regulator known to be associated with tumorigenesis. Oncogenic transcription factor MYC (c-Myc) is upregulated in one-third of breast cancers. The aim of this study is to evaluate the correlation between expression of proteins involved in cell cycle regulation and oncogenesis with occurrence of distant metastasis in triple and non-triple negative breast cancer.

Design: Breast cancer cases with adequate clinical-pathologic follow-up information were retrieved between 2005 and 2013. Tissue microarrays were constructed using 1.0 mm cores, with 3 cores obtained per case. Immunohistochemistry for Cyclin D1, p16, p21/

WAF1 and p27 /KiP1, TP53, BCL2, and c-MYC was performed. Allred scoring system was used for nuclear reactivity. Cases with at least one scorable core were considered evaluable. Follow up information was collected via patient electronic medical records and the Cancer Registry. Logistic regression was used to calculate statistical significance; p-value <0.05 was considered significant.

Results: There were 188 evaluable cases. Overexpression of Cyclin D1 was directly associated with likelihood of distance metastasis, while overexpression of p16 was inversely associated with distant metastasis in non-triple negative breast cancer (Table 1a). There was no significant association between evaluated promitotic and antimitotic proteins and distant metastasis in triple negative breast cancer (Table 1b).

N=140	Analysis of maximum likelihood estimates	Odds ratio (95% confidence limits)	Correlation to Distant metastasis (p value)
CCND1 (Cyclin D1)	0.1663	1.181 (1.001-1.394)	0.0492
CDKN2A (p16)	-0.2421	0.785 (0.656-0.939)	0.0080
CDKN1A (p21)	0.1304	1.139 (0.909-1.428)	0.2579
CDKN1B (p27)	0.1532	1.166 (0.980-1.386)	0.0827
TP53 (p53)	-0.0502	0.951 (0.821-1.101)	0.5020
BCL2	0.0262	1.027 (0.884-1.192)	0.7320
MYC (c-Myc)	-0.1086	0.897 (0.738-1.090)	0.2754

Table 1a. Logistic regression of correlation between promitotic and antimitotic proteins with breast cancer distant metastasis for non-triple negative phenotype

N=48	Analysis of maximum likelihood estimates	Odds ratio (95% confidence limits)	Correlation to Distant metastasis (p value)
CCND1 (Cyclin D1)	0.00925	1.009 (0779-1.307)	0.9441
CDKN2A (p16)	0.0503	1.052 (0.878-1.260)	0.5854
CDKN1A (p21)	0.2555	1.291 (0.838-1.989)	0.2467
CDKN1B (p27)	0.0324	1.033 (0.801-1.331)	0.8026
TP53 (p53)	0.0837	1.087 (0.932-1.269)	0.2886
BCL2	-0.0328	0.968 (0.772-1.213)	0.7757
MYC (c-Myc)	0.1612	1.175 (0.946-1.459)	0.1441

Table 1b. Logistic regression of correlation between promitotic and antimitotic proteins with breast cancer distant metastasis for triple negative phenotype

Conclusions: Overexpression of CDKI like p16 or promitotic protein like Cyclin D1 may prove to be useful markers in helping to predict clinical outcome in non-triple negative breast cancer. However, none of the cell cycle regulator proteins evaluated were observed to be significantly helpful in predicting outcome for triple negative breast cancer.

302 Correlation Between Cyclin-Dependent Kinase Inhibitors and Promitotic Proteins with Overall Survival in Breast Cancer Patients

Jaime Singh[1], *Nitai Mukhopadhyay*[2], *Michael Idowu*[3]
[1]Virginia Commonwealth University Health Systems, Richmond, VA, [2]VCU Health Systems, [3]Virginia Commonwealth Univ Health System, Richmond, VA

Background: Breast cancer is the most common cancer accounting for about 30% of new cancer diagnosis in women. While survival has improved with early diagnosis and advancement in treatment, breast cancer is still the second most common cause of death in women. The recent FDA approval of palbociclib, a selective inhibitor of cyclin dependent kinase (CDK) 4 and CDK6 has brought renewed focus on cell cycle regulators, especially CDK inhibitors (CDKI). CDKI like CDKN2A (p16), CDKN1A (p21) and CDKN1B (p27) are believed prevent the CDK4/6-Cyclin D1 complex, thereby limiting progression of tumor cells from G1 to S phase. Cyclin D1 forms a complex with CDK4 and CDK6 to aid progression of tumor cells from G1 to S phase. The primary objective of this study was to evaluate the correlation between expression of some CDKI and promitotic protein expression, with overall survival of breast cancer patients. Our secondary objective was to evaluate the association of race, body mass index and some proteins known to be associated with tumorigenesis or tumor suppression with overall survival.

Design: Breast cancer cases with adequate clinical-pathologic follow-up information were retrieved between 2005 and 2013. Follow up information including race, BMI, and overall survival was collected via patient electronic medical records and the Cancer Registry. Patients lost to follow-up or with missing follow-up information were excluded from analysis. Tissue microarrays were constructed using 1.0 mm cores, with 3 cores obtained per case. Immunohistochemistry for CCND1 (Cyclin D1), CDKN2A (p16), CDKN1A (p21)/WAF1 and CDKN1B (p27)/KiP1, TP53 (p53), BCL2, and MYC (c-MYC) was performed. Allred scoring system was used for nuclear reactivity. Cases with at least one scorable core were considered evaluable. Cox Proportional Hazards Model was fitted using SAS v9.4 to evaluate time to event analysis with overall survival. P-value <0.05 was considered significant.

Results: There were 193 evaluable cases. The results are shown in the Table 1.

	Analysis of Maximum Likelihood Estimates	Hazard ratio (95% confidence limits)	Time to Event Analysis with Overall Survival (p-value)	Comment (Negative likelihood estimates and Hazard Ratio less than 1.0 with a significant p-value means longer survival or positive outcome)
Race (Black vs White)	-0.64238	1.901 (1.161-3.112)	0.0106	The hazard ratio for survival or positive outcome is almost double for white compared to black
BMI	0.00250	1.003 (0.968-1.038)	0.8885	No correlation to overall survival
CCND1 (cyclin D1)	-0.12206	0.885 (0.810-0.967)	0.0069	Possibly due to response to Rx – tumor going through cell cycle and dividing
CDKN2A (p16)	0.05801	1.060 (0.979-1.147)	0.1516	No correlation to overall survival
CDKN1A (p21)	-0.01815	0.982 (0.843-1.144)	0.8162	No correlation to overall survival
CDKN1B (p27)	-0.13369	0.875 (0.790-0.969)	0.0104	Possibly due to inhibition of CDK4/CDK6 preventing transition from G1 to S phase of the cell cycle
TP53 (p53)	0.04807	1.049 (0.972-1.133)	0.2186	No correlation to overall survival
BCL2	-0.11189	0.894 (0.829-0.965)	0.0038	Possibly due to response to Rx – tumor going through cell cycle and dividing
MYC (c-Myc)	-0.02269	0.978 (0.879-1.087)	0.6760	No correlation to overall survival

Table 1. Cox Proportional Hazard Model of correlation between CDKI and promitotic proteins, BMI and race with survival

Conclusions: Overexpression of CDKI like p27 showed better overall survival; other CDKI do not have significant association with overall survival. Overexpression of promitotic protein like Cyclin D1 and anti-apoptotic protein like BCL2 is associated with better overall survival, possibly due to increased response to treatment of proliferating tumor cells. While there is significant racial disparities in overall survival, BMI showed no significant association.

303 Clinico-Pathologic and Molecular Profile of Invasive Lobular Carcinoma with Extracellular Mucin (ILCEM)

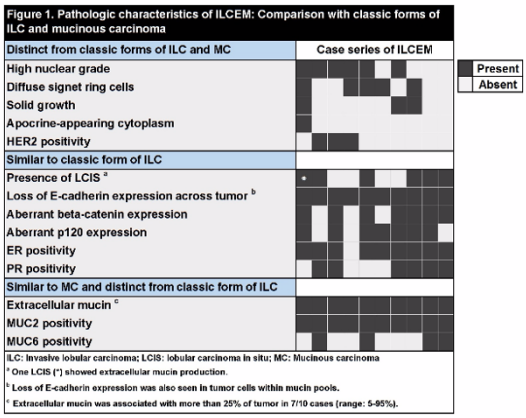
Thing Rinda Soong[1], *Deborah Dillon*[2], *Tad Wieczorek*[3], *Laura Collins*[4], *Susan Lester*[5], *Stuart Schnitt*[6], *Beth T Harrison*[1]
[1]Brigham and Women's Hospital, Boston, MA, [2]Brigham and Women's Hospital, [3]Brigham and Women's Hospital, Boston, MA, [4]Beth Israel Deaconess Medical Center, Boston, MA, [5]Brigham & Women's Hospital, Boston, MA, [6]Brigham and Women's Hospital; Dana Farber Cancer Institute, Boston, MA

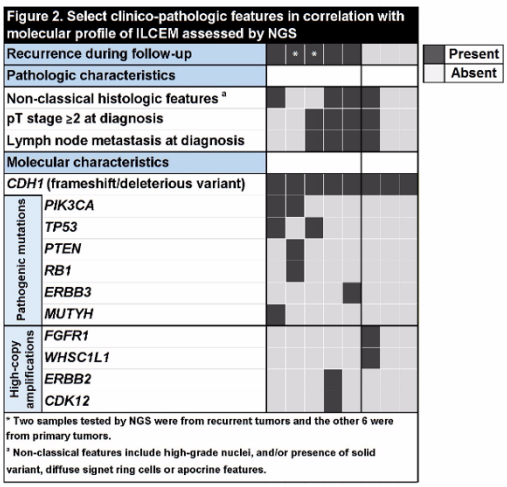
Background: ILCEM is a rare breast cancer subtype not recognized by the WHO. Only 13 cases have been previously reported, with the majority described as ER+/HER2- and grade 1-2 carcinomas with lobular morphology and variable extracellular mucin. Little is known about the pathologic or genomic signatures that might distinguish ILCEM from classic forms of invasive lobular carcinoma (ILC) or conventional mucinous carcinoma.

Design: We studied 10 breast cancers with lobular histomorphology and extracellular mucin production diagnosed at our institution between 2001-2017. Eight tumors with sufficient tissue for DNA extraction were further analyzed by a hybrid-capture next generation sequencing (NGS) assay that interrogates the full coding sequences of 447 genes for mutations and copy number variations (CNVs).

Results: Median patient age was 69 yrs (range: 31-77 yrs). Nine cases presented with a radiologic mass (1 palpable), of which 5 were >2 cm and had lymph node metastases. All cases were moderately (7) or poorly differentiated (3), frequently exhibiting non-classical morphology that has not been previously described or emphasized

(**Figure 1**), including grade 3 nuclei (6), signet ring cells (5), solid growth (3), or apocrine features (1). All tumors displayed MUC2 positivity and loss of E-cadherin expression. Concurrent LCIS was seen in 6/10 cases, 1 of which showed extracellular mucin associated with the LCIS. Receptor profiles were ER+/HER2- (7), ER+/HER2+ (2) and ER-/HER2+ (1). With a median follow-up of 36 months (range:3-97 months), 5 patients had recurrences (4 distant; 1 locoregional) resulting in 2 cancer-related deaths. The most common CNVs were 1q gain with concurrent 16q loss (6), 22q loss (5) or 18q loss (5). Genes with recurrent CNVs included loss of *TP53* (6), *CBFB* (5), gain of *RUNX1* (3) and *FOXA1* (3). Frameshift/deleterious variants of *CDH1* were detected in all cases, with *POLQ* missense mutations enriched in 1/3 of tested tumors. Most cases with recurrences (4) were identified with pathogenic variants including *PIK3CA* (2), *PTEN* (1), *TP53* (2), *MUTYH* (1), *ERBB3* (1) mutations, or biallelic loss of *RB1* (1) (**Figure 2**).





Conclusions: To our knowledge, this is the first and largest series of ILCEM with clinico-pathologic and genomic analyses, highlighting it as a distinct variant of ILC that often presents with higher-stage and non-classical features. NGS data support an overall lobular-type molecular profile, and reveal potentially targetable alterations in a subset of cases with recurrence.

304 CD68 and CD163 Predict Recurrence and Progression of Breast Ductal Carcinoma in situ

Aye Aye Thike[1], *Valerie Koh*[2], *Nur Diyana Md Nasir*[1], *Puay Hoon Tan*[3]. [1]Singapore General Hospital, [2]Singapore, [3]Singapore

Background: Ductal carcinoma *in situ* (DCIS) of the breast, also known as pre-invasive cancer, is heterogeneous clinically, morphologically and genetically. With mammography being widely available for breast cancer screening, DCIS accounts for 20–25% of newly diagnosed breast cancers. Although women with pure breast DCIS have excellent prognosis, those with subsequent invasive recurrence have an increased risk of breast cancer-specific death. Tumor-associated macrophages (TAMs) are closely involved in tumorigenesis by inducing angiogenesis and invasion. We aimed to investigate the role of TAM markers CD68 and CD163 in predicting DCIS recurrence and progression.

Design: The cohort comprised 198 DCIS cases consisting of 101 tumors with known recurrent disease and 97 non-recurrent DCIS as controls. Immunohistochemistry was performed on standard sections using antibodies to ER, PR and HER2 to define triple negativity, CK14, EGFR and 34 E12 to detect basal-like phenotype, CD68 and CD163. Positive biomarker expression of TAM markers was defined as membranous and cytoplasmic staining of 10% or more of either tumor cells or surrounding immune infiltrates. Disease free survival (DFS) was defined as time from diagnosis to recurrence or date of last follow up, and correlated with TAM markers.

Results: CD68 and CD163 expression in immune infiltrates surrounding DCIS was observed in 19% and 24% of cases respectively. Tumor cells did not express TAM markers. Higher nuclear grade was significantly associated with both CD68 and CD163 expression ($p<0.001$ and $p=0.001$ respectively). CD163 expression was statistically associated with presence of microinvasion ($p=0.004$) and basal-like DCIS ($p=0.028$). On Kaplan-Meier analysis, unfavorable DFS for ipsilateral recurrence and progression was observed in patients whose tumors harbored high expression of both CD68 ($p=0.004$) and CD163 ($p=0.024$). Higher CD163 expression had impact on ipsilateral invasive recurrences ($p=0.001$). On multivariate analysis, tumors harboring high CD163 expression independently predicted both DCIS recurrence and progression (95%CI 1.214-6.791, HR 2.871, $p=0.016$).

Conclusions: Our study demonstrates that increased TAM expression in immune infiltrates in the DCIS environment was associated with poorer prognostic parameters, and predicted DCIS recurrence and progression. Further characterization of the DCIS immune microenvironment may identify high risk patients for stratified treatment.

305 CXCR4 is a Potential Imaging and Therapeutic Target in Basal-Like Breast Cancer

Jon Thomason[1], *Stephanie Chen*[1], *Andrew Bellizzi*[1]. [1]University of Iowa Hospitals and Clinics, Iowa City, IA

Background: The chemokine receptor CXCR4 has numerous roles in health and disease: its natural ligand CXCL12 causes stem cells to home to the bone marrow, anti-CXCR4 therapy mobilizes stem cells, germline mutations cause primary immunodeficiency (WHIM syndrome), sporadic mutations are found in 30-40% of lymphoplasmacytic lymphomas, and it serves as a co-receptor for T-cell-tropic HIV strains. CXCR4 is widely expressed by cancer, though many previous reports of immunohistochemical expression have used suboptimal protocols (PMID: 19116653). CXCR4-based nuclear medicine imaging and peptide receptor radionuclide therapy are in development, and our group has a clinical and research interest in managing cancer patients with these modalities. One of our goals is to use CXCR4 immunohistochemistry (IHC) to aid in patient selection.

Design: CXCR4 IHC (clone UMB-2; 1:250) was performed on tissue microarrays constructed from 181 breast cancers (triplicate 1 mm cores). Expression was evaluated in terms of intensity (0, 1+, 2+, 3+) and extent (0-100%) with an H-score (intensity*extent) calculated. Vital status and results of clinical ER/PR/HER2 testing were recorded with an intrinsic subtype (i.e., luminal A, luminal B/HER2-, luminal B/HER2+, Erb-B2 overexpression, basal-like) inferred according to 2013 St. Gallen Criteria. Fisher's exact test was used with $p<0.05$ considered significant.

Results: CXCR4 expression was seen in 17% of basal-like breast cancers and uncommonly in other intrinsic subtypes (see Table). CXCR4-positivity was significantly more frequent in basal-like cancers than in the combined remainder ($p=0.0027$). CXCR4+ and CXCR4- basal-like cancer patients were equally likely to be alive (57% and 55%) ($p=1$).

Table: CXCR4 Expression Stratified by Intrinsic Subtype

Intrinsic Subtype	% CXCR4+	Mean (Median) H-score (if +)
Luminal A (n=23)	4%	20 (20)
Luminal B/HER2- (n=27)	7%	8 (8)
Luminal B/HER2+ (n=4)	0%	NA
Erb-B2 overexpressor (n=37)	0%	NA
Basal-like (n=90)	17%	41 (23)

Conclusions: CXCR4 is more frequently expressed in basal-like breast cancers than in other intrinsic subtypes. Given relatively limited clinical options in the treatment of this disease, anti-CXCR4 therapy, especially in the setting of primary resistance or recurrence, could be applied. IHC with the UMB-2 monoclonal antibody is well-positioned to select patients for CXCR4-based imaging or therapy.

306 Androgen Receptor is Frequently Expressed by ER and HER2-Positive Breast Cancers but is Largely Restricted to Carcinomas with Apocrine Features among Triple-Negative/Basal-Like Breast Cancers Limiting its Predictive Value

Jon Thomason[1], *Neha Dhungana*[2], *Kristin C Jensen*[3], *Andrew Bellizzi*[1]. [1]University of Iowa Hospitals and Clinics, Iowa City, IA, [2]University of Iowa Hospitals and Clinics, [3]Stanford Hospital, Stanford, CA

Background: We recently validated immunohistochemistry for androgen receptor (AR) as a diagnostic marker for sebaceous, salivary duct, and prostatic carcinomas and as a possible predictive marker in metastatic AR-expressing neoplasms. Aromatase inhibitors, either singly or in combination with other agents, are used in metastatic ER+/HER2- breast cancer. Androgen ablation would be potentially applicable to AR+, ER/PR/HER2- breast cancers.

Design: Tissue microarrays were constructed from 178 breast cancers (triplicate 1 mm cores). AR immunohistochemistry was performed with expression evaluated in terms of intensity (0, 1+, 2+, 3+) and extent (0-100%) with an H-score (intensity*extent) calculated. An H-score >/=1 was considered positive. Vital status and results of clinical ER/PR/HER2 testing were recorded with an intrinsic subtype (i.e., luminal A, luminal B/HER2-, luminal B/HER2+, Erb-B2 overexpression, basal-like) inferred according to 2013 St. Gallen Criteria. AR+ basal-like cancers were evaluated for the presence of apocrine features. Mann-Whitney and Fisher's exact tests were used with p<0.05 considered significant.

Results: AR-expression correlated with intrinsic subtype, with frequent expression in ER+, less frequent expression in ER-/HER2+, and infrequent expression in basal-like cancers (see Table). AR-positivity was significantly less frequent in basal-like cancers than in the combined remainder (p<0.0001). AR-expression was more extensive in luminal A than luminal B cancers (p=0.0002). Apocrine features were noted on the H&E of 91% of AR+ basal-like cancers (prominent in 64%). AR+ and AR- basal-like cancer patients were equally likely to be alive (55% each) (p=1). AR+ Erb-B2 overexpressing patients were more likely to be alive (68% vs 54% for AR-), but the result was not significant (p=0.48).

Table: AR Expression Stratified by Intrinsic Subtype

Intrinsic Subtype	% AR+	Mean (Median) H-score (if +)
Luminal A (n=22)	77%	191 (193)
Luminal B/HER2- (n=27)	74%	66 (31)
Luminal B/HER2+ (n=4)	75%	145 (138)
Erb-B2 overexpressor (n=36)	61%	94 (58)
Basal-like (n=89)	12%	166 (180)

Conclusions: AR is frequently expressed by ER+ and Erb-B2 overexpressing breast cancers. In basal-like breast cancers, expression appears largely restricted to carcinomas with apocrine features, limiting apparent applicability of aromatase inhibitor therapy to this small subset.

307 Evaluation of the BRCAness phenotype and its correlations with clinicopathologic features in triple-negative breast cancers

Tian Tian[1], *Ling Shan*[1], *Wentao Yang*[2], *Xiaoyan Zhou*[1], *Ruohong Shui*[2]. [1]Fudan University Shanghai Cancer Center, Shanghai, [2]Fudan University Shanghai Cancer Center, Shanghai, China, Shanghai Medical College, Fudan University, Shanghai, China, Shanghai

Background: Some sporadic triple-negative breast cancers (TNBCs) share similar clinicopathologic and molecular characteristics with BRCA1/2-mutant breast cancers, a phenotype described as "BRCAness". Identifying BRCAness in TNBCs can expand the target group for platinum salts and PARP inhibitors. The aim of our study was to assess the clinical validity of BRCA1/2 promoter methylation and BRCA1-like genomic profile to identify BRCAness, and to evaluate its correlations with clinicopathologic features in TNBCs.

Design: Formalin-fixed, paraffin-embedded (FFPE) tissues and fresh tissues of 151 primary invasive TNBCs were collected. BRCA1/2 germline mutations of the 151 TNBCs had been detected by next-generation sequencing. BRCA1/2 promoter methylation and BRCA1-like genomic profile were detected using Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) and MLPA assay respectively. BRCA1/2 mRNA expression were evaluated by quantitative reverse transcriptase polymerase chain reaction. The expression of CK5/6, epidermal growth factor receptor (EGFR), and Ki67 proteins were assessed using immunohistochemistry.

Results: 38 (25.2%) of 151 cases showed BRCA1 promoter methylation and no methylation was found in BRCA2 promoter. 31 (25%) of 124 cases had a BRCA1-like MLPA profile. BRCA1-germline mutations and BRCA1 promoter methylation were mutually exclusive events (P = 0.002). In the 31 cases with BRCA1-like MLPA profile, 8 cases exhibited BRCA1 promoter methylation and 6 cases carried BRCA1/2 germline mutation. BRCA1 promoter methylation was significantly associated with low BRCA1 mRNA expression (P < 0.0001), grade 3 tumor (P = 0.03), high Ki67 levels (P = 0.02) and basal-like breast cancers (BLBCs) (P = 0.0001). BRCA1-like profile was significantly correlated with large tumor size (> 2 cm, P = 0.02) and BLBCs(P = 0.006). The BRCAness phenotype was significantly associated with large tumor size (> 2 cm, P = 0.009), positive lymph nodes (P = 0.008), grade 3 tumor (P = 0.0001), high Ki67 levels (P = 0.001) and BLBCs (P = 0.0001).

Conclusions: Combined detection of BRCA1 promoter methylation and BRCA1-like genomic profile could identify more BRCAness cases in TNBCs. BRCA1 promoter methylation might be used to rule out BRCA1-mutant breast cancers. The BRCAness phenotype was associated with aggressive clinicopathologic features. These findings were important to both hereditary breast cancer screening and target treatment of TNBCs.

308 Lymphocytic Mastopathy: A Seventeen-Year Clinicopathologic Review of an Uncommon Breast Lesion

Vanda Torous[1], *Hilde Vardeh*[2], *Liza Quintana*[3]. [1]Beth Israel Deaconess Medical Center, Boston, MA, [2]Beth Israel Deaconess Medical Center, Boston, MA, [3]Beth Israel Deaconess Medical Center, Boston, MA

Background: Lymphocytic mastopathy (LM), also known as diabetic mastopathy, is an uncommon lesion that has been described in patients with longstanding type 1 diabetes mellitus (DM) as well as other autoimmune diseases (AD). It is characterized by the combination of dense, keloidal-like fibrosis; periductal, perilobular, and perivascular lymphocytic infiltrates; and stromal epithelioid myofibroblasts. While the microscopic features of LM have been described, the natural history of this lesion remains vague, as well as the specificity of the histologic findings.

Design: A retrospective review of our institution's pathology laboratory information system was conducted to identify cases in which a diagnosis of LM was made or suggested from 1/2000 to 8/2017. 91 cases from 57 patients were identified and H&E stained slides of these cases were reviewed by two breast pathologists. A clinicoradiologic review of the medical record was then performed.

Results: Cases from 40 patients demonstrated all of the histologic features of LM and had sufficient clinical history available, with mean follow-up time of 6 years. 52.5% had DM, 12.5% AD, and 35% neither DM nor AD (Table 1). 75% presented with a palpable mass, most commonly single and unilateral. Imaging findings were variable, although the majority were BIRADS 4A or less (57.5%). The diagnostic procedures included 35% core needle biopsy (CNB) alone, 10% CNB or fine-needle aspiration (FNA) then CNB, 35% CNB or FNA then excision, and 20% excisional biopsy alone. There were no notable differences in histologic features between patients with DM, AD, or those without either. A single case had incidental atypical lobular hyperplasia. 32.5% (13/40) of patients had persistent or recurrent lesions radiologically. Of these, 38% (5/13) were biopsied, all of which were consistent with LM.

Table 1. Demographic, Clinicoradiologic, and Outcome Data of 40 Lymphocytic Mastopathy Cases

Female	97.5% (39/40)
Age (median)	40.5 years (range 22-81)
Diabetes	52.5% (21/40)
Type 1 Diabetes	71% (15/21)
Type 2 Diabetes	29% (6/21)
With Concomitant Autoimmune Disease	40% (10/21)
Autoimmune Disease Only	12.5% (5/40)
Neither Diabetes nor Autoimmune Disease	35% (14/40)
Presentation	
Palpated mass	75% (30/40)
Routine imaging	17.5% (7/40)
Other symptoms (nipple discharge, tenderness)	7.5% (3/40)
Single mass	80% (32/40)
Unilateral	82.5% (33/40)
Subareolar location	45% (18/40)
Imaging Findings (BIRADS Score)	
1 (Negative ie palpable mass only)	25% (10/40)
3 (Probably benign)	2.5% (1/40)
4A (Low suspicion for malignancy)	30% (12/40)
4B (Intermediate suspicion for malignancy)	30% (12/40)
4C (Moderate concern for malignancy)	12.5% (5/40)
Diagnostic Procedure	
CNB only	35% (14/40)
CNB or FNA then CNB	10% (4/40)
CNB or FNA then excision	35% (14/40)
Excisional biopsy only	20% (8/40)
Recurrence	
Yes	32.5% (13/40)
Follow-Up Biopsy	12.5% (5/40)

Abbreviations: CNB=core needle biopsy, FNA=fine needle aspiration

Conclusions: We herein have described to our knowledge the largest series of cases of LM at one institution. Patients tend to present with a single, palpable mass. While most patients have DM or AD (65%), a third of our cohort did not have either. Thus, the classic histologic features of LM are not entirely specific for these diseases. Our data support the benign nature of LM and suggest that cases with classic features of LM, regardless of known DM or AD, may be amenable to clinicoradiologic follow-up in order to decrease the number of excisional biopsies and repeat procedures.

309 A Comparison of PD-L1 mRNA In Situ Hybridization and Immunohistochemistry in 241 Primary Breast Carcinomas

Emily A Towery[1], *Erik Dill*[2], *Anne Mills*[3]. [1]University of Virginia, [2]UVA Medical Center, Charlottesville, VA, [3]Charlottesville, VA

Background: Immunotherapy targeting the PD-1/PD-L1 axis has shown promise in a subset of breast carcinomas, particularly among high-grade triple negative cancers (TNBC). However, the relationship between PD-L1 expression by immunohistochemistry (IHC) and treatment vulnerability is unclear. Experience from other organs suggests that IHC expression is an imperfect biomarker for treatment response, with a subset of PD-L1-positive tumors showing no significant therapeutic benefit and another group of PD-L1-negative tumors responding despite absent expression. Chromogenic in situ hybridization (ISH) is a clinically available methodology to detect mRNA and may represent a useful biomarker in selecting optimal candidates for immunotherapy.

Design: PD-L1 IHC (Ventana SP142, internally calibrated against Dako 22C3) and mRNA ISH (RNAScope, Advanced Cell Diagnostics) were performed on 241 primary breast cancers on tissue microarray (4 replicate cores per case). IHC and ISH were both considered positive with ≥1% of tumor cells staining. IHC positivity was based on circumferential membranous staining and ISH positivity was based on dot-like cytoplasmic +/- nuclear staining. Pathologic data including grade, stage, and hormone receptor/HER2 status were collected. Statistics were performed using a two-tailed Z test.

Results: PD-L1 IHC and mRNA ISH were positive in 11.6% (28/241) and 18.3% (44/241) of all cases, respectively, including 32.1% (18/56) and 44.6% (25/56) of TNBC. TNBC were more likely to be positive for PD-L1 IHC ($p<0.0002$) and PD-L1 mRNA ISH ($p<0.0002$) compared to non-TNBC. There were 23 ISH-positive, IHC-negative cases. Conversely, there were 7 IHC-positive, ISH-negative cases, all of which showed >10% tumor cell staining, and 4 of which were >25% positive.

	PD-L1 IHC		PD-L1 mRNA ISH	
Grade*	1: 2.04% 2: 2.70% 3: 30.77%	(1/49) (3/111) (24/78)	1: 4.08% 2: 10.81% 3: 38.46%	(2/49) (12/111) (30/78)
Stage**	1: 12.23% 2: 13.16% 3: 5.56% 4: 0.00%	(17/139) (10/76) (1/18) (0/2)	1: 20.14% 2: 17.11% 3: 16.67% 4: 0.00%	(28/139) (13/76) (3/18) (0/2)
Triple negative	Y: 32.14% N: 5.41%	(18/56) (10/185)	Y: 44.64% N: 10.27%	(25/56) (19/185)

*3 and **6 cases excluded because of incomplete/unavailable data

Conclusions: PD-L1 mRNA ISH is positive in a greater number of breast cancers than PD-L1 IHC, with particularly high expression among TNBC. mRNA ISH may therefore highlight a population of tumors developing incipient immune resistance that could be amenable to PD-1/PD-L1 inhibitors even in the absence of IHC expression. A subset of cases are negative for mRNA ISH and positive for IHC; given that these cases typically show high level PD-L1 IHC staining, these may represent cancers with compensatory mRNA down-regulation in the setting of high protein expression. Clinical trials are needed to determine whether mRNA ISH represents a predictive biomarker for anti-PD-1/PD-L1 response in breast cancers, particularly among TNBC.

310 A Combination of GATA3 and SOX10 is Useful for the Diagnosis of Metastatic Triple Negative Breast Cancer

Gary Tozbikian[1], *Debra Zynger*[2]. [1]The Ohio State University, Columbus, OH, [2]The Ohio State University Medical Center, Columbus, OH

Background: It can be difficult to establish the origin of a breast metastasis if the primary tumor is triple negative breast cancer (TNBC) or if there is a loss of biomarker expression in the metastasis. Many breast lineage markers show reduced sensitivity in TNBC. SOX10 expression has been reported in primary TNBC but is poorly studied in metastatic lesions. In this study, we evaluated the diagnostic utility of a panel of SOX10, GATA3, and androgen receptor (AR) in metastatic breast cancer negative for ER, PR, and HER2 (MBC) and compared the expression of these markers to the matched primary breast cancer (PBC).

Design: We conducted a retrospective search to identify MBC diagnosed from 2013-17 that lacked expression of ER, PR, and HER2 and had available PBC specimens (n=34). The PBC was ER+/HER2- in 12, ER+/HER2+ in 1, and triple negative in 21 cases. The frequency of immunohistochemical expression of GATA3, SOX10, and AR was assessed.

Results: In the MBC, 82% were positive for GATA3, 59% for SOX10, and 26% for AR. Nearly all MBC (97%) were positive for either GATA3 or SOX10, with 44% dual positive, and only 1 case negative for both markers. Most GATA3 negative MBC cases were SOX10 positive (83%). AR expression was only seen in GATA3 positive MBC (47%), and was significantly more frequent in SOX10 negative MBC (50%) vs. SOX10 positive MBC (10%, p=0.02). Overall concordance for GATA3, SOX10, and AR between the PBC and metastasis was 89%, 74%, and 84% respectively.

Conclusions: While GATA3 is a more sensitive lineage marker than SOX10 in MBC, SOX10 is a useful adjunct because it is positive in the majority of GATA3 negative breast metastases. Utilizing both GATA3 and SOX10 is recommended for confirming breast as the site of origin in metastases that lack ER, PR, and HER2 expression whereas the addition of AR is not helpful.

311 A Novel Detection Methodology for HER2 Protein Quantitation in Clinical Samples: Correlation with Pathologic Response to Trastuzumab-Based Neoadjuvant Therapy.

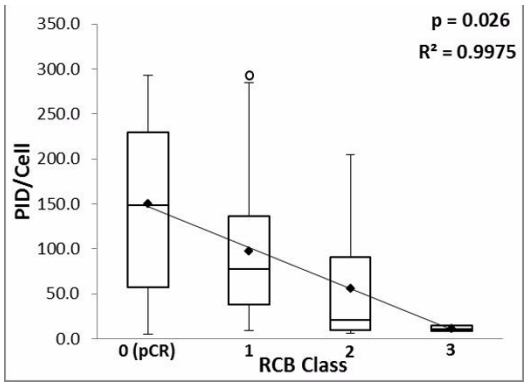
Bradley M. Turner[1], *Brandon Buscaglia*[2], *Hideki Goda*[3], *Loralee Mcmahon*[2], *Takako Natori*[3], *Hisatake Okada*[4], *Armen Soukiazian*[5], *YASUSHI NAKANO*[6], *David Hicks*[7]. [1]University of Rochester Medical Center, Rochester, NY, [2]University of Rochester Medical Center, [3]Konica Minolta INC., Hino, Tokyo, [4]Konica Minolta INC., Hino-city, Tokyo, [5]University of Rochester Medical Center, Rochester, NY, [6]Konica Minolta,Inc., Tokyo, [7]Rochester, NY

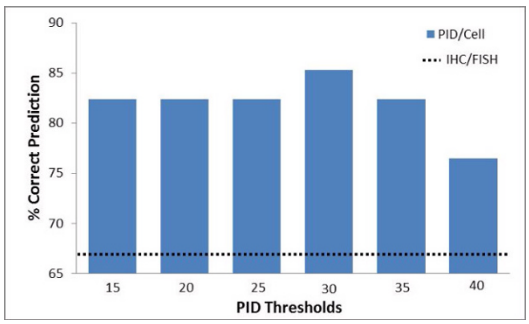
Background: The over-expression of Human Epidermal growth factor Receptor-2 (HER2) in breast cancers is associated with a poor prognosis. Targeting HER2-overexpression has been shown to be a remarkably effective therapeutic modality. Current clinical assays to assess HER2 status include immunohistochemistry (IHC) to detect protein over-expression and fluorescence in situ hybridization (FISH) to detect gene amplification. Both IHC and FISH methodologies have limitations. Given that the target of currently approved drugs is the HER2 receptor protein, detection systems that quantitatively measure HER2 protein may be useful. In the current study, we have employed a novel detection methodology using streptavidin-coated Phosphor Integrated Dot fluorescent nanoparticles (PID) to quantitatively measure HER2 protein expression in the pretreatment biopsies from 34 HER2-positive carcinomas (determined by IHC and/or FISH) that had undergone neoadjuvant Trastuzumab-based chemotherapy. Measurement of HER2 protein was correlated with the pathologic response to treatment in the post therapy resection.

Design: Clinical pathologic variables for thirty-four cases of HER2-positive breast cancer that had undergone neoadjuvant chemotherapy plus HER2-targeted therapy were retrieved from the medical record database at URMC, including the post-treatment residual cancer burden (RCB) score and ER/PR/HER2 status. HER2 protein can be quantitatively measured by PID immunofluorescence using computer assisted image analysis. Quantitative assessment of HER2 protein was determined on the pre-treatment biopsy and correlated with the post-treatment residual cancer burden (RCB) class.

Results: Specimen demographic data is represented in Table 1. Measurement of PID/cell correlated significantly with RCB class (Figure 1, $R^2=0.99$). We hypothesized a positive predictive threshold of 30 PID/cell for a good pathologic response (pCR or RCB class 1). Using this threshold of 30 PID/cell, we correctly predicted the pathologic response in 29 out of 34 cases (85.3%, Figure 2) versus 23 out of 34 cases (67.6%, Figure 2) for IHC and/or FISH analysis.

Parameter	n (Average)	% (Range)
N Total	34	
Age	(54)	(23-83)
Diagnosis (DX)		
Invasive Ductal Carcinoma	33	97.1
Invasive Lobular Carcinoma	1	2.9
Nuclear Grade		
1	2	5.9
2	7	20.6
3	25	73.5
ER/PR Status		
ER+/PR+	21	61.8
ER+/PR-	2	5.9
ER-/PR+	0	0
ER-/PR-	11	32.4
HER2 IHC Score		
0	1	2.9
1+	0	0
2+	10	29.4
3+	23	67.6
Residual Cancer Burden Class (RCB)		
0 (pCR)	12	35.3
1	11	32.4
2	8	23.5
3	3	8.8





Conclusions: Quantitative measurement of HER2 protein using PID nanoparticles demonstrate great potential for the assessment of HER2-protein in breast cancer samples, and may be useful for predicting pathologic response to Trastuzumab-based neoadjuvant therapy. Further studies in a larger patient cohort are warranted.

312 Microsatellite Instability and PD-1/PD-L1 Expression in HER2-Positive Breast Carcinoma

Julianne M Ubago[1], *Alexandra Larson*[2], *Lauren Rosen*[2], *Jorge Novo*[3], *Jennifer Pincus*[4], *Luis Blanco*[5], *K. P. Siziopikou*[1]. [1]Northwestern University, Chicago, IL, [2]Chicago, IL, [3]Northwestern University Feinberg School of Medicine, Chicago, IL, [4]Northwestern Memorial Hospital, Chicago, IL, [5]Northwestern University Feinberg School of Medicine, Chicago, IL

Background: The PD-1/PD-L1 pathway represents a new therapeutic target for many solid tumors, especially in immunogenic tumor subtypes. Recently, the FDA approved a PD-L1 inhibitor for use in any solid tumor showing microsatellite instability (MSI) regardless of tissue origin or histologic subtype. HER2 amplification is found in 20-30% of breast carcinomas and is associated with a poor prognosis. While treatment with anti-HER2 therapies has improved outcomes, a subset develop resistance and require novel therapeutic options. In addition, HER2+ breast tumors have higher levels of tumor infiltrating lymphocytes (TILs) and are reported to be highly immunogenic. Our group previously reported PD-1/PD-L1 expression in HER+ breast carcinoma. In this study, we evaluated MSI in HER2+ breast carcinomas and correlated it with the PD-1 and PD-L1 expression in both the tumor cells and TILs.

Design: The study population consisted of 114 patients with HER2+ invasive breast carcinoma diagnosed from 2009-2014 (mean age 53, range 18-80). Tissue microarrays were constructed (3 cores/case) and evaluated for the immunohistochemical mismatch repair (MMR) proteins MLH1, PMS2, MSH2, and MSH6 and scored as positive or negative. These results were then compared with PD-1 and PD-L1 expression in tumor cells and TILs which were evaluated using a composite score based on extent and intensity of staining (range 0-9; 0-3=negative, 4-9=positive).

Results: Overall, 5 of 114 cases (4.4%) showed complete loss of either the MLH1/PMS2 or the MSH2/MSH6 MMR pair and four of these were grade 3 tumors. Three cases showed loss of both MLH1/PMS2 and 2 showed loss of both MSH2/MSH6. In addition, one case showed loss of only MLH1. Of the 5 cases, one showed PD-L1 expression in tumor cells, one showed PD-L1 expression in TILs, one showed both PD-L1 and PD-1 expression in TILs, and one showed only PD-1 expression in TILs. The fifth case showed no significant PD-1 or PD-L1 expression.

Conclusions: 1. MSI is present in a small subset of HER2+ breast carcinomas. **2.** The majority of these cases are grade 3 tumors. **3.** Of the MSI tumors, one showed significant PD-L1 expression in the tumor cells and three showed expression of either PD-L1 or PD-1 in the TILs. These findings raise the possibility that it may be beneficial to perform a MMR panel in refractory cases of HER2+ breast cancer in order to potentially utilize alternative immunomodulatory therapies.

313 Pseudoangiomatous Stromal Hyperplasia as the Sole Pathologic Diagnosis on Breast Core Needle Biopsy: Correlation with Radiologic Findings and Clinicoradiologic Outcomes.

Hilde Vardeh[1], *Alexander Pyden*[2], *Vanda Torous*[3], *Tejas Mehta*[4], *Laura Collins*[2], *Liza Quintana*[2]. [1]Beth Israel Deaconess Medical Center, Boston, MA, [2]Beth Israel Deaconess Medical Center, Boston, MA, [3]Beth Israel Deaconess Medical Center, Boston MA, [4]Newton, MA

Background: Pseudoangiomatous Stromal Hyperplasia (PASH) of the breast is a benign stromal change. Historically uncommon, the diagnosis of PASH has become more frequent due to the introduction of widespread screening mammography and higher resolution imaging, as well as increased awareness of this entity by pathologists. The optimal management of PASH has been debated, and surgical excision continues to be performed without data supporting this rationale. Herein we demonstrate that with adequate biopsy sampling of the lesion and radiologic-pathologic concordance, a diagnosis of PASH does not require surgical intervention, or imaging follow-up.

Design: We retrospectively reviewed the data of 95 consecutive patients with PASH as the sole pathologic diagnosis on breast core needle biopsy (CNB). All patients were female. All CNBs were originally interpreted by breast pathologists (7/2010- 7/2016) in an academic hospital. We reviewed the extent of pathologic involvement by PASH in the CNBs, and analyzed reported diagnostic imaging findings, management recommendations, and clinicoradiologic outcomes through online medical records review.

Results: The median patient age was 43 years (range 19-78). 31 patients (33%) presented with a palpable mass, 37 (39%) were detected on screening imaging, and 27 (28%) on diagnostic imaging for follow-up of preexisting conditions. Imaging findings of a mass were seen in 78 (82%). On pathologic review of the CNBs, 71 (75%) had diffuse involvement by PASH. Radiologic-pathologic concordance was established with high level of certainty in 85 patients (89%) which required no imaging follow-up. The remaining 10 (11%) were considered most likely concordant with recommendation of imaging follow-up. Clinicoradiologic follow-up data were available for 53 (56%) of the patients and mean follow-up duration was 33.8 months (SD 19.7). 7 patients had repeat biopsies of the area of PASH, and 2 had a subsequent excision. None of the patients had a BI-RADS upgrade on imaging, and there were no upgrades on pathology with re-biopsy or excision.

Conclusions: To our knowledge this is the largest case series of breast CNBs with PASH as the sole pathologic diagnosis with follow-up data. Our findings support the benign nature of PASH, and underscore the importance of a multidisciplinary radiologic-pathologic approach in diagnostic work-up of breast CNBs in order to lower the rates of unnecessary surgical intervention and the costs of redundant imaging follow-up.

314 Clinical and Pathologic Features of Invasive Breast Cancers with OncotypeDx Recurrence Score Less Than 11

Poonam Vohra[1], *Gregor Krings*[2], *Tianming Chu*[3], *Yunn-Yi Chen*[4]. [1]UCSF, San Francisco, CA, [2]UCSF, San Francisco, CA, [3]UCSF, [4]Univ. of California, San Francisco, San Francisco, CA

Background: Identification of invasive breast cancer (IBC) with low recurrence potential may prevent overtreatment. According to the 2018 AJCC staging guidelines, patients with hormone receptor positive, HER2 negative, lymph node (LN) negative tumors and OncotypeDx recurrence score (RS) less than 11 are staged as T1a-T1b regardless of T size. It is unclear whether IBC with RS<11 (designated here as ultra-low RS, ULRS) demonstrate specific pathologic features and/or biomarker profile. We aimed to study the clinical, morphological and immunophenotypic features in this unique subset of IBC.

Design: All IBC with ULRS over a 12-year period were identified. Of 133 patients with ULRS, 42 had LN metastases and were excluded. Of the remaining 91 cases, 73 had slides available for review and constituted the cohort. Age, histologic subtype, stage, Nottingham grade, ER, PR, HER2, Ki-67 index, and IHC4 score were evaluated. Treatment and outcomes were reviewed to determine the frequency and type of adverse events.

Results: Mean age was 56 years (range 36-74). Most tumors were invasive ductal carcinoma (IDC; 60/73, 81%), with remaining subtypes including invasive lobular carcinoma (ILC; 8 including 1 pleomorphic ILC, 11%), IBC with ductal and lobular features (3), micropapillary carcinoma (2), IBC with solid papillary features (1), tubular carcinoma (1), mucinous carcinoma (1) and IDC with associated encapsulated papillary carcinoma (1). Most (93%) IBC were grade 1 (38%) or 2 (55%). All IBC were ER positive and HER negative, with mean ER H-score of 260 (range 98-300). The mean Ki-67 index was 7.7% (range 0.5-40%) and mean IHC4 score was -24.4 (range -106.4 to +105.6). Most (66%) IBC were T1 (27% T2, 5% T3). Follow-up was available for 72 (99%) cases (mean=52, range 1-140 months). Treatment included chemotherapy (8%), hormone therapy (76%) and radiation (40%). Six patients (8%) had local recurrence, 1 of which also had distant metastasis.

Conclusions: IBC with ULRS are mostly low or intermediate grade IDC but include grade 3 tumors and other subtypes such as micropapillary carcinoma and pleomorphic ILC. Although most have high ER H-score, low Ki-67 index and low IHC4 score, some show low ER expression, high Ki-67 index and high IHC4 score. These tumors may recur or metastasize despite ULRS.

315 Comprehensive Molecular Profiling of Pleomorphic Ductal Carcinoma of the Breast with Theranostic Implications

Semir Vranic[1], *Juan Palazzo*[2], *Jeffrey Swensen*[3], *Joanne Xiu*[3], *Elena T Florento*[4], *Zoran Gatalica*[5]. [1]College of Medicine, Qatar University, Doha, Qatar, [2]Thomas Jefferson Medical College, Philadelphia, PA, [3]Caris Life Sciences, [4]Caris Life Sciences, Phoenix, AZ, [5]Caris Life Sciences, Phoenix, AZ

Disclosures: Jeffrey Swensen: *Employee*, Caris Life Sciences Joanne Xiu: *Employee,* Caris Life Sciences

Background: Pleomorphic ductal carcinoma (PDC) is a very rare subtype of invasive ductal carcinoma, characterized by the presence of highly atypical/bizarre (>6-fold variation in nuclear size) and multinucleated giant neoplastic cells comprising >50% of the tumor cell population. PDC is typically triple-negative breast cancer (TNBC), associated with a poor outcome.

Design: Formalin-fixed paraffin-embedded tissue samples of the 6 PDC patients were tested with NextSeq platform (592-gene panel). Tumor mutational load (TML) was calculated using only low frequency germline and somatic nonsynonymous missense mutations; microsatellite instability (MSI) analysis was performed by looking at repeat tracts sequenced in the 592-gene panel. ArcherDx FusionPlex Assay was used to detect gene fusions (52 gene targets). Immunohistochemistry was used to detect expression of ER, PR, Her2 and PD-L1 in tumor cells.

Results: All PDCs were negative for ER, PR and Her2 expression. *TP53* mutations were detected in 5/6 cases, with one case harboring two additional pathogenic mutations (*SMARCA4* R1093X and *FH* K477dup), and two cases with pathogenic *BRCA1* (E143X) or *KRAS* (G12A) mutations. No pathogenic mutation was detected in one case. TML was low in all cases (range 4-11/Mb) and no MSI-H was detected in any case. No gene fusions were detected. Gene amplification of Cyclin Dependent Kinase Inhibitor 1B (*CDKN1B*) and Fibroblast Growth Factor Receptor 1 (*FGFR1*) were detected in one case, each. Tumor expression of PD-L1 (tumor proportion score, TPS) was negative in two or low (<50%) in three cases, while immune cells (IC) expressing PD-L1 were detected at potentially significant levels (≥5%, IC2) in 2 cases (with TPS-L).

Conclusions: PDCs exhibit significantly less targetable gene alterations in contrast to related TNBC and metaplastic carcinomas previously analyzed by our group; single case with a mutation in *BRCA1* indicated potential benefit to platinum-compounds and PARP inhibitors. Furthermore, a low TML, rare PD-L1 expression and absence of mismatch repair deficiency make this tumor an unlikely candidate for treatment with immune check point inhibitors.

316 Revisiting Lobular Histology and Risk of Bilateral Breast Cancer in the Current Era: a Contemporary Assessment

Carol Wang[1], *Zuzana Kos*[2]. [1]University of Ottawa, Ottawa, ON, [2]Kanata, ON

Background: Current dogma holds that lobular histology is associated with a higher rate of bilaterality than ductal carcinomas. Due to its infiltrative growth, mammogram often underestimates the extent of lobular carcinomas. In our institution, breast MRI is standard when biopsy reveals invasive lobular carcinoma, whereas in the case of invasive ductal carcinoma, use of MRI is at the surgeon's discretion. MRI leads to an increased rate of biopsy for benign breast changes. We investigated whether in the current era of mammographic screening and early detection, lobular histology remains a risk factor for synchronous bilateral breast cancer.

Design: All consecutive invasive breast cancer resection cases between July 2011 to January 2017 were retrieved from our pathology electronic database. Clinicopathologic data were extracted, including histologic subtype and presence of bilateral disease. Patients with prior breast cancer were excluded.

Results: 3382 patients met inclusion criteria. In the study period, the overall incidence of invasive lobular carcinoma remained stable, ranging from 11.3-12.9% per year of all invasive breast cancer diagnoses. 93 (2.7%) patients had synchronous bilateral invasive breast cancer (defined as diagnosed on initial imaging or workup of the index cancer). The incidence of lobular histology (in one or both breasts) in women with bilateral breast cancer was significantly higher at 33.8% (p<0.00001). Bilateral carcinoma was identified on initial mammogram in 40 patients (43% of bilateral cases). The contralateral cancer was diagnosed on MRI on workup of the index cancer in 48 (51%) of patients. In these patients, the contralateral carcinoma was significantly smaller (average size 1.1 cm, range 0.1-4.7 cm) than the index cancer (average size 3.6 cm, range 0.4-14 cm). In 41.7% of patients with contralateral cancer diagnosed on MRI, the index tumour was of lobular type. The contralateral (MRI detected/mammogram occult) cancer was of lobular type in 25% of patients. In 5 (5%) of patients, the contralateral cancer was found in a contralateral prophylactic mastectomy (3) or balancing reduction (2).

Conclusions: Lobular histology remains a significant risk factor for synchronous bilateral breast cancer although the majority of bilateral cases are of ductal histotype. MRI identifies contralateral breast cancers that are occult on mammogram and at a lower stage than the index cancer. This supports the importance of contralateral breast screening at the time of primary diagnosis.

317 Epithelial Cell Adhesion Molecule (EpCAM) Expression in Invasive and in situ Lobular Carcinomas

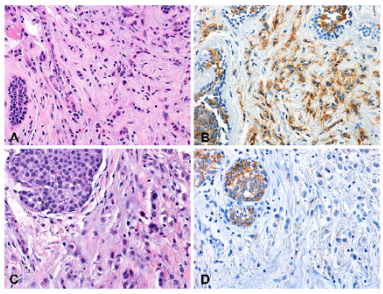
Elizabeth Yiru Wu[1], *Rajni Sharma*[2], *Peter Illei*[3], *Pedram Argani*[4], *Ashley Cimino-Mathews*[5]. [1]Brigham and Women's Hospital, Boston, MA, [2]Johns Hopkins Medical Institutions, Baltimore, MD, [3]Johns Hopkins University School of Medicine, Baltimore, MD, [4]Johns Hopkins Hospital, Baltimore, MD, [5]Johns Hopkins Hospital, Baltimore, MD

Background: Epithelial cell adhesion molecule (EpCAM) has been shown to antagonize cell-cell interactions mediated by E-cadherin. Clinically, higher levels of EpCAM expression is associated with poor prognosis in patients with many carcinoma types, including invasive ductal carcinoma (IDC), and EpCAM has been investigated as a therapeutic target. Invasive lobular carcinoma (ILC) is characterized by dysfunction of E-cadherin, and an early study reported lower EpCAM positivity in ILC (59%) than IDC (81%) (J Clin Pathol 2011;64:415-20). Here, we evaluate the relationship of EpCAM and clinicopathologic features in ILC, as well as the relationship of EpCAM expression in lobular carcinoma in situ (LCIS) with ILC.

Design: Tissue microarrays containing 46 evaluable primary ILC were labeled for EpCAM by immunohistochemistry; 23 tumors had associated LCIS. EpCAM labeling was scored as percentage (0, 1=1-9%; 2=10-49%; 3=50-79%; 4=80-100%) and intensity (0-3) labeling; the percentage and intensity scores were multiplied to classify tumors as having low EpCAM expression (composite score 1-3) or high EpCAM expression (composite score 4-12).

Results: EpCAM labeled 56% of ILC, and 24% displayed high expression (**Fig 1A-B**). High EpCAM expression was significantly associated with higher stage at presentation; 100% of patients with high EpCAM presented with stage II-IV disease, in contrast to 60% of patients with negative-low EpCAM (p=0.02). ILC with high EpCAM tended to be larger (mean 3.8 cm vs. 2.6 cm; p=0.09), higher grade

(27% grade III vs. 17% grade III, p=0.31), more often triple negative (27% vs. 6%, p=0.08) and in younger patients (mean 56 years vs. 61 years, p=0.28) than ILC with negative-low EpCAM. EpCAM labeled 74% of LCIS, and 48% displayed high expression (in contrast to 24% of ILC, p=0.058). The majority of LCIS and associated ILC displayed concordant EpCAM; however, a significant minority (35%) were discordant. Of these, the majority (63%) were EpCAM^{+} LCIS with EpCAM^{-} (**Fig 1C-D**).



Conclusions: A subset of ILC shows high EpCAM expression, and EpCAM is a potential therapeutic target in this difficult-to-treat tumor type. ILC with high EpCAM display unfavorable clinicopathologic features, and EpCAM may serve as a marker of tumors with more aggressive features. The observation that a subset of tumors display EpCAM loss from the LCIS to the ILC suggests that EpCAM loss contributes to the development of invasive disease; further investigation into the ways EpCAM may modulate tumor biology is warranted.

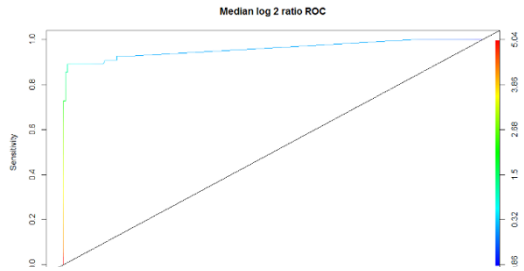
318 Clinical Utility of HER2/ERBB2 Copy Number Analysis by Next Generation Sequencing in Metastatic Breast Cancer

Daniel Xia[1], *Frank Kuo*[2], *Deborah Dillon*[2]. [1]University Health Network, Toronto, ON, [2]Brigham & Women's Hospital, Boston, MA

Background: A combination of immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) is the standard of care for HER2 evaluation in breast cancer. In this study, we investigate the utility of next generation sequencing (NGS)-derived *HER2/ERBB2* copy number (CN) analysis by correlating NGS data with HER2 IHC/FISH results in metastatic breast cancer.

Design: Metastatic breast cancer cases previously tested by OncoPanel (a targeted hybrid-capture-based NGS assay for solid tumors) and HER2 IHC/FISH were included. Correlation analyses focused on the median *ERBB2* log-2 ratios and interval counts (the number of consecutive intervals on chromosome 17 showing copy number change) from OncoPanel, average *HER2* CN and *HER2/CEP17* ratios from FISH, and the HER2 IHC scores. We specifically sought to determine the log-2 ratio cutoffs that most reliably predict positive and negative IHC/FISH results. HER2 positive was defined as IHC 3+ or *HER2/CEP17* ratio ≥2.0 and *HER2* copy number ≥6.0 and HER2 negative was defined as IHC 0 or 1+ or *HER2/CEP17* ratio <2.0 and *HER2* copy number <4.0. Analyses were performed using R.

Results: 299 metastatic breast cancers were evaluated, including 57 HER2 positive cancers, 211 HER2 negative cancers, and 31 less common HER2 copy number combinations (e.g. positive with ratio < 2.0 and *HER2 CN ≥ 6.0,* low positive results and equivocal results). Overall, the *ERBB2* log-2 ratios from the OncoPanel NGS assay were strongly correlated with HER2 results from IHC/FISH (receiver operator curve area = 0.96; see Figure 1). *ERBB2* log-2 ratio ≥1.5 had 100% positive predictive value for a HER2 positive result by IHC/FISH and a log-2 ratio <0.0 had 100% negative predictive value for a HER2 negative result by IHC/FISH. Additionally, we found the interval counts on NGS to correlate with HER2 positive (median = 32; interquartile range [IQR] 28 to 37 intervals) and negative results (median = 114; IQR 55 to 227.5 intervals).



Conclusions: This study establishes specific log-2 ratio cutoff points on OncoPanel that strongly predict positive and negative HER2 status as determined by standard of care methods. Positive and negative results by IHC/FISH also correlate strongly with interval counts.

319 Efficient and Robust Cell Segmentation in Breast Microscopy Image using Fully Convolutional Neural Network with Multi-Context Aggregation

Yuanpu Xie[1], *Pingjun Chen*[1], *Jinzheng Cai*[2], *Fuyong Xing*[1], *Lin Yang*[1]. [1]University of Florida, [2]University of Florida, Gainesville, FL

Background: Accurate cell detection and segmentation serve as critical prerequisites for subsequent biomedical image analysis. While they still remain challenging due to inhomogeneous background, touching cells, and large variations in cell size and shape. Moreover, the ever-increasing amount of available dataset and high-resolution of whole slide images pose further demand for fast cell detection and segmentation algorithms. In this abstract, we present an efficient and robust cell centroids guided segmentation method.

Design: We propose a multi-context aggregation fully convolutional neural network that can be applied to both detection and segmentation task by only adjusting the last layer and loss function. First, classification network is used to segment cell foreground from background. Then, we apply the structured regression network to detect cell centroids. At last, detected cell centroids are used as seeds to guide the post-processing for separating touching cells in segmented foreground. The proposed network can not only integrate multi-level context information, but also preserve the high-resolution semantic information for accurate cell detection and segmentation. Its graphical illustration is shown in **Fig.1**. The proposed method is robust to highly inhomogeneous background noise and dense cell touching. It is designed with the consideration of efficiency, thus can be applied to whole slide image analysis.

Results: We evaluate our method on the challenging breast cancer dataset. The automatic segmentation results are measured by Dice Similarity Coefficient (DSC), Hausdorff Distance (HD), and Mean Absolute Distance (MAD) in comparision with state-of-the-art. We report the quantitative results in **Table.1**, and also show representative qualitative results in **Fig.2**.

Comparative pixel-wise segmentation accuracy on breast cancer. For each metric (DSC, HD, MAD), the mean, median, standard deviation (STD), and the sorted 80% highest accuracy among all the results are listed.

Method	DSC				HD				MAD			
	Mean	Medi-an	Std	80%	Mean	Medi-an	Std	80%	Mean	Medi-an	Std	80%
MS	0.49	0.51	0.25	0.74	20.83	17.77	15.59	28.70	13.17	11.74	8.27	18.63
ISO	0.56	0.58	0.19	0.75	17.19	15.40	10.54	24.36	11.59	10.38	5.82	15.89
SUP	0.68	0.72	0.18	0.84	17.14	13.89	13.10	24.72	9.32	7.74	6.02	13.29
GCC	0.59	0.62	0.23	0.81	16.84	15.59	10.40	23.91	10.75	9.43	6.57	15.64
MWS	0.73	0.79	0.20	0.88	11.12	9.66	7.25	14.80	6.66	5.08	5.49	9.24
RLS	0.77	0.81	0.15	0.88	10.05	8.12	7.46	14.76	6.30	4.80	4.95	8.25
CNN-SP	0.80	0.86	0.15	0.92	8.60	6.37	6.77	13.08	6.24	4.53	4.90	9.61
Ours	0.80	0.85	0.15	0.91	10.28	6.78	8.64	16.41	5.99	4.15	4.85	8.73

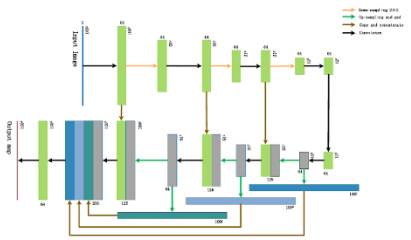


Fig 2: Multi-context aggregation fully convolutional neural network architecture (Please note that the input image size does not need to be fixed). The green or gray box denote the feature maps, the number of feature map channel is marked on top or bottom of each box. The size of feature maps (along row and column dimension) is denoted on the right-hand side of each box. Different operations are denoted using arrows with different colors. For

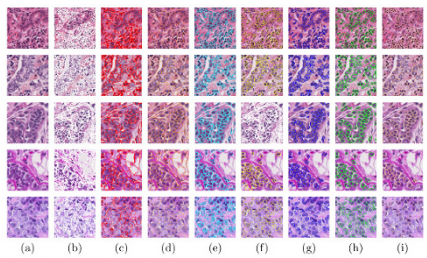


Fig 5: Cell segmentation results using different methods on several breast cancer image patches. (a) Original images, (b) MS, (c) ISO, (d) SUP, (e) GCC, (f) MWS, (g) RLS, (h) CNN-SP and (i) our method. It is clear to see our method can deal with touching cells and inhomogeneous background noise. It achieves comparable results to the state-of-the-art with much shorter running time.

Conclusions: Our results demonstrate two orders of magnitude faster in speed and competitive performance in accuracy over state-of-the-art.

320 The Prognosis Significance of Micropapillary Feature in Pure Mucinous Carcinoma of the Breast

Xiaoli Xu[1], *Wentao Yang*[2]. [1]Fudan University Shanghai Cancer Center, Fudan University Shanghai Medical College, Shanghai, [2]Fudan University Shanghai Cancer Center, Shanghai, China, Shanghai Medical College, Fudan University, Shanghai, China, Shanghai

Background: Pure mucinous carcinoma (PMC) of the breast is a low grade breast carcinoma with indolent biological behavior. Invasive micropapillary carcinoma represents a tumor with high rate of lymphovascular invasion and lymph node metastasis. Micropapillary structure was also observed in PMC and the prognostic significance of micropapillary feature in PMC of the breast remains in debate.

Design: 75 cases of breast PMC operated and diagnosed in FDACC during 2007-2010 were collected. Sentinel lymph node biopsy or axillary lymph nodes dissection was performed in 74 cases. Clinicopathological features including age, sex, tumor size, growth pattern, nuclear grade, lymph node status, and immunohistochemistry(IHC) of hormone receptors, HER2, Ki-67 proliferation index were analyzed. Uncertain HER2 staining was verified by fluorescence in situ hybridization (FISH). 71 PMC were followed up from 18-110 months (median 68 months). All data was analyzed by SPSS statistic software.

Results: All PMC patients were female, age from 31-83 years (median 57 years). Tumor size was from 0.5-5.5cm (median 2.2 cm) in diameter. 45 cases (60%) were nuclear grade 1, 30 cases (40%) were grade 2. ER positive was 100%, PR was 91%. No HER2 3+ was detected. 4 uncertain HER2 cases showed no gene amplification by FISH. 64 (89.3%) cases had <20% Ki-67 index. Only 4 cases (5.7%) had lymph node metastasis. Micropapillae with varied percentage were observed in 60 (80%) PMC. 5 groups were divided according to the percentage of micropapillae of 0%, <20%, 20-49%, 50-90% and >90%. There were 15(19.2%), 14(18.7%), 18(24%), 17(22.7%) and 11(14.7%) cases in each group. The follow-up results showed no recurrence or distant metastasis. Statistic analysis revealed only tumor size related to lymph node metastasis significantly($P<0.05$). Micropapillary feature, no matter the percentage, had no significant relationship with lymph node metastasis and survival status.

Conclusions: Our data suggests that PMC is a low grade breast carcinoma with excellent prognosis. Micropapillae with low/intermediate nuclear grade is common in PMC, it has no significant relationship with lymph node metastasis and survival.

321 Increased PD-L1 Expression in HER2-Positive Breast Cancer without Pathologic Complete Response to Neoadjuvant Therapy

Fei Yang[1], *Juhee Song*[2], *BARBARA MINO*[3], *Edwin R Parra*[1], *Aysegul Sahin*[4], *Ignacio Wistuba*[5], *Jennifer Litton*[2], *Elizabeth Mittendorf*[6], *Yun Wu*[6]. [1]UT MD Anderson Cancer Center, Houston, TX, [2]M.D. Anderson Cancer Center, [3]UT-MD Anderson Cancer Center, Houston, TX, [4]MD Anderson Cancer Center, Houston, TX, [5]M. D. Anderson Cancer Center, Houston, TX, [6]Houston, TX

Background: Neoadjuvant chemotherapy combined with trastuzumab significantly improves pathologic complete response (pCR) in the HER2-positive breast cancer. However, there is still a fraction of HER2-positive breast cancer patients that do not experience a pCR. This study was performed to evaluate PD-L1 expression after the neoadjuvant therapy in non-pCR patients to identify potential strategies for further treatment.

Design: Using chromogenic immunohistochemistry (IHC) and multiplex immunofluorescence (mIF), PD-L1 protein was measured in the pre-treated core needle biopsy (CNB) and paired surgical specimen. 21 non-pCR HER2-positive breast cancer patients were enrolled. PD-L1 IHC staining (clone-E1L3N) was evaluated via Aperio digital pathology software. H-scores in the tumor nests and stroma were reported separately. A mIF panel of 6 biomarkers (AE1/AE3, PD-L1, PD-1, CD3, CD8, and CD68) was used to confirm PD-L1 expression and identify cell type of PD-L1 positive cells. mIF staining was performed using Opal™ 7-color Kit and scanned using the Vectra Multispectral Imaging System version 3.0 (Perkin Elmer), and analyzed using InForm image analysis software (InForm™ 2.3.0, PerkinElmer). Associations between the tumor and stroma PD-L1 levels and clinical-pathological variables were analyzed.

Results: PD-L1 was detected in both tumor cells and immune cells with a predominant membranous staining pattern. There was a statistically significant increase in PD-L1 expression in residual disease compared with the CNB sample. The post PD-L1 expression in the both stroma and tumor was significantly greater than pre PD-L1 expression [stroma PD-L1 median (IQR): 9.0 (6-16) *vs.* 1.35 (1-3), $p<0.0001$; tumor PD-L1 median (IQR): 2 (1-4) *vs.* 1 (1-1), $p=0.0473$]. When evaluated by the presence of TIL post-treatment, the increase in PD-L1 stroma expression was greater with a borderline significance in tumors with >/=50% TIL than <50% TIL [40 (11-99) *vs.* 6.5 (2-13), $p=0.0625$]. Five surgical cases with the highest PD-L1 H-score in the stroma were stained by mIF. PD-L1 positive macrophages identified by colocalization of PD-L1 and CD68 expressing cells were found both in the stroma and tumor cell nests in all 5 cases.

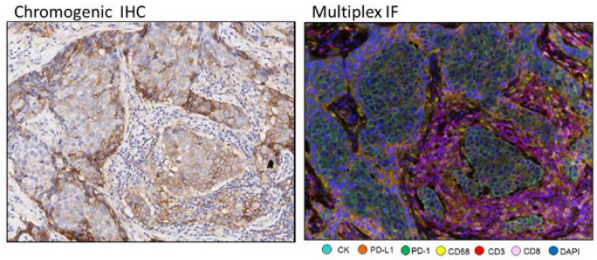


Figure1. PD-L1 expression in the HER2-positive breast cancers by chromogenic IHC and multiplex IF

Conclusions: PD-L1 protein was increased in the residual tumor from HER2-positive breast cancer patients after neoadjuvant therapy. These data suggest a potential strategy in targeting PD-L1 therapeutically in the HER2-positive breast cancer patients resisted on neoadjuvant systemic therapy.

322 Development of a Revolutionary Tissue-Based Assay That permits Simultaneous Co-Localization of Any Protein and Any gene in Breast Cancer in Diagnostically Challenging Settings

Hadi Yaziji[1], *Siba El Hussein*[2], *Lucia Amador*[3], *Dianelis Gonzalez*[4], *Albert Alhatem*[5], *Robert Poppiti*[6], *Carlos Genty*[7]. [1]Vitro Molecular Laboratories, LLC, South Miami, FL, [2]Mount Sinai Medical Center, Miami Beach, FL, [3]Vitro Molecular Laboratories, [4]Vitro Molecular Laboratories, LLC, Miami, FL, [5]University of Aleppo, Aleppo, Syria, [6]Mount Sinai Hospital, Miami Beach, FL, [7]Critical X Solutions

Background: FISH has been widely used in breast cancer, especially for HER2 testing. Given the dark ligh conditions associated with FISH, there are settings in which FISH interpretation is difficult. Examples include small tumor volume, admixture of in-situ and invasive disease, admixture of cellular benign lesion and invasive disease, lymph node micrometastasis, and tumors within vascular spaces. It is challenging in such scenarios to reliably find/select the tumor area for interpretation. If there is a means to target specific proteins exclusively expressed by the tumor cells or adjacent cells in tissue sections, and if these proteins can be detected immunofluorescently simultaneously alongside gene FISH probes, such challenges we constantly face in real-life settings can be easily overcome.

Design: We currently optimized a combined immunofluorescence and FISH procedure for simultaneous detection of any protein (with available targeting antibodies) and any gene (with available FISH probes). 30 invasive breast cancers were specifically selected to include the above-described challenging settings. The antibody/probe combinations include p63/HER2, GATA3/HER2, Cytokeratin/HER2, ERG/HER2 to localize myoepithelial cells, rare mammary tumor cells (GATA3), rare mammary tumor cells (Keratin) and endothelial cells, respectively. The immunofluorescence detection is performed using a sensitive detection technology. Directly conjugated, fluorescently labeled FISH probes are separately applied on the same section using the same routine FISH assay. Assay precision is accomplish with 5 rounds of testing. Parallel immunohistochemistry and FISH studies were also performed on the same tumors for signal comparison, using the same antibody clones and FISH probes.

Results: In all 30 cases, tumor nests of interest were easily and definitively isolated by co-localizing the protein of interest and HER2 gene. The protein signal is readily visualized even with lower magnifications (10X and 20X), and was comparable to the immunostained sections. The FISH signal intensity is comparable between the pure and hybrid FISH assays.

Conclusions: This study demonstrates the power of this assay to fluorescently co-detect any protein alongside FISH (HER2 gene), making it possible to overcome post-analytical challenges of FISH interpretation, which improves interpretation accuracy. The applications of this assay are also broad and can be applied in any subspecialty in anatomic pathology requiring accurate target localization.

323 A Multiple Breast Cancer Stem Cell Model to Predict Recurrence of T1-3, N0 Breast Cancer

Feng Ye[1], *Yan Qiu*[1], *Hong BU*[1]. [1]West China Hospital, Sichuan University, Chengdu, Sichuan, China

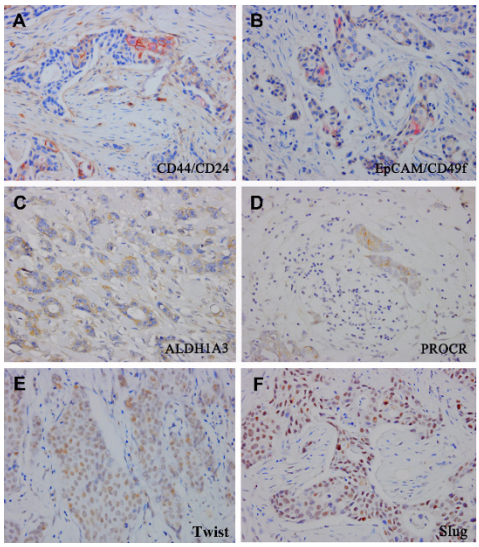
Background: Local or distant relapse is the key event for the overall survival (OS) of early-stage breast invasive ductal carcinoma (EBIDC) after initial surgery. As the breast cancer stem cells (BCSC) were proven resistant for therapy and contributed to recurrence, we tried to develop and validate a prognostic model for EBIDC recurrence based on the prevalence of multiple BCSCs.

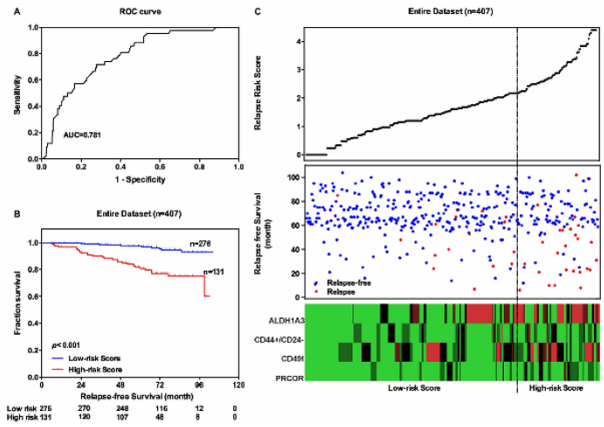
Design: Immunochemistry (IHC) and dual IHC were performed to identify and quantify the BCSCs. Clinicopathological characteristics of 407 breast cancer patients diagnosed at 2006-2011 in West China Hospital were analyzed. The performance of Cox proportional hazard regression model was assessed using the holdout method, where the dataset was randomly split into two exclusive sets (70% training and 30% testing sets). Additionally, we performed bootstrapping to overcome a possible biased error estimate and obtain confidence intervals (CI). Combined univariate cox proportional model and bootstrapping, four biomarkers (ALDH1A3, $CD44^{+}/CD24^{-}$, CD49f, PROCR) were identified significantly associated with relapse free survival (RFS), and integrated as a prognostic panel to calculate a recurrence score (named RRS) and to determine a risk group (high or low) for each patients.

Results: Among the 407 patients, 67.81% and 32.19% patients were categorized into low-risk and high-risk groups according to the RRS. The Kaplan-Meier estimates of the relapse rate at 5 years in the low-risk group (2.67%, 95% CI: 0.72%-4.63%) was significantly lower than that in high-risk group (19.30%, 95% CI: 12.34%-26.27%) ($p<0.001$).

Table 1. Kaplan-Meier Estimation of the Rate of Recurrence at 5 Years, According to Recurrence-Score Risk Category.

RRS		Percentage of patients (%)	Rate of recurrence at 5 years (95% CI)#	**p**-value
Training set	Low-risk	67.54	2.32 (2.00-2.63)	< 0.001
	High-risk	32.46	18.67 (17.84-19.50)	
Testing set	Low-risk	68.46	3.18 (2.24-4.12)	< 0.001
	High-risk	31.54	17.87 (15.67-20.07)	





Conclusions: In the multiple Cox model, RRS provided significant classifier power and was independent of age at diagnosis and tumor size ($p<0.001$). Besides, we found that in ER-positive group with high-risk score, no matter hormonal therapy was performed or not, there is no significant difference in RFS ($p=0.668$).

324 Quantitative Imaging and Deep Profiling of Collagen Structure of Asian Triple Negative Breast Cancer: Novel Perspective for Breast Cancer Micro-Environment

Joe Yeong[1], *Laurent Gole*[2], *Jeffrey Chun Tatt Lim*[3], *Kok Haur Ong*[2], *Aye Aye Thike*[4], *Kiat Hon Lim*[5], *Jabed Iqbal*[3], *Bernett Lee*[6], *Weimiao Yu*[2], *Puay Hoon Tan*[7]. [1]Singapore General Hospital, Singapore, [2]Institute of Molecule and Cell Biology, A*STAR, Singapore, [3]Singapore General Hospital, Singapore, [4]Singapore General Hospital, [5]Singapore General Hospital, Singapore, [6]Singapore Immunology Network (SIgN), Agency of Science, Technology and Research (A*STAR), Singapore, [7]Division of Pathology, Singapore General Hospital, Singapore

Background: Tumor stromal biology has been less studied compared to the cancer cell compartment and the immune cell population, until recent findings highlighted the indispensable influence of stromal reaction to the efficacy of treatment including immunotherapy. Collagen fibers are the major structural extracellular matrix component in breast and other tumours, and increased stromal collagen fibers have been found to facilitate breast tumour formation, invasion, and metastasis.

Design: Stromal remodeling is featured by collagen realignment in the stromal compartment, collagen fibers and basal membrane, which can be identified, quantified and visualized by state-of-the-art stain-free pathology imaging technologies Second Harmonic Generation and Two Photon Emission (SHG-TPE). We profiled 388 triple-negative breast cancer (TNBC) patients with 68 SHG-TPE parameters and correlate with clinicopathological parameters.

Results: Among all the parameters, high Collagen Fiber Density (CFD) showed marginal association with better disease free survival and overall survival (DFS p=0.047; OS p= 0.044). Furthermore, we developed algorithms to further differentiate the collagen fibers into "aggregated collagen fibres" and "thin collagen fibres" based on the complexity and texture of the fibers. Interestingly only aggregated CFD is associated with better prognosis (DFS and OS, p=0.014; p= 0.033) but not thin CFD. On the other hand, we found that every 1 percent increment of aggregated/thin collagen ratio was associated with better DFS (HR 0.97, 95% CI 0.96-1.00, p= 0.015). The addition of aggregated/thin collagen ratio to clinicopthological features significantly increased the prognostic value for DFS (ΔLR^2= 8.4, p=0.004), compared to clinicopathological features alone (histological grade, tumour size and lymph node status).

Conclusions: Our automated image analysis pipeline with SHG-TPE offers a novel platform to quantitate stromal collagens of the tumor microenvironment to better predict the clinical outcome of patients with triple negative breast cancer. With breast cancer as proof-of-concept for our findings, we plan to apply and validate in other cancers, potentially translating to clinical applications in the imminent future.

325 Impact Of New Prognostic Staging System In Breast Cancer On Traditional Anatomic Stage System

Esther Yoon[1], *Christopher J Schwartz*[2], *Farbod Darvishian*[3]. [1]New York University Medical Center, New York, NY, [2]New York University Medical Center, New York, NY, [3]New York University Medical Center, New York, NY

Background: Traditionally, the American Joint Committee on Cancer (AJCC) staging uses Tumor size, Node status and distant Metastasis (TNM) to determine breast cancer prognosis, known as anatomic stage (AS). In the eighth edition, new parallel prognostic stage (PS) groups incorporate tumor biomarkers such as estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), tumor grade and Recurrence Score (RS). The new PS system attempts to refine the prognostic groups tailored to the tumor biology. Starting 2018, both AS and PS will be available to patients. In anticipation of the change, we performed a one-year retrospective study to look at how the breast cancer cases would be staged according to the new PS compared to the traditional AS.

Design: Patients diagnosed with primary breast cancer in 2016 were identified using pathology laboratory software system. Only the cases with positive ER, PR and negative HER2 and concurrent Oncotype Dx RS were included. The cases were reviewed and stratified using the traditional TNM AS groups and also into the new PS groups. The change of each case's AS and PS was analyzed.

Results: 106 cases were reviewed; 27 cases had Oncotype Dx RS less than 11 and 79 had equal to and greater than 11. Most common AS and PS were IA (67 and 65 cases, respectively). PS remained unchanged in 58.5% of cases, of which 91% were stage IA. Seventeen (16%) cases were upstaged and 27 (25%) cases were downstaged (see table 1).

AS	Cases (%)	PS	Cases (%)	Upstaged	Cases (%)	Downstaged	Cases (%)
IA	67 (63.2%)	IA	65 (61.3%)	From IA to IB	11 (10.4%)		
IB	4 (3.8%)	IB	29 (27.4%)			From IB to IA	4 (3.8%)
IIA	29 (27.4%)	IIA	5 (4.7%)	From IIA to IIIA	1 (0.9%)	From IIA to 1A	4 (3.8%)
						From IIA to 1B	18 (16.9%)
IIB	6 (5.7%)	IIB	1 (0.9%)	From IIB to IIIA	3 (2.8%)	From IIB to IB	1 (0.9%)
				From IIB to IIIB	2 (1.9%)		
IIIA	0	IIIA	4 (3.8%)				
IIIB	0	IIIB	2 (1.9%)				
	106		106		17 (16.0%)		27 (25.4%)

Table 1. Summary of stages of 106 cases and PS changes when using 2018 AJCC biomarker parameters

Conclusions: The new PS staging will bring about significant change to the traditional AS system-nearly half (42%) of cases reviewed at our institution would see a change in new prognostic stage grouping. We, as pathologists, need to reach a consensus as to how to integrate the results of the ancillary tests into our pathology reports in preparation for the upcoming PS to better serve our breast cancer patients.

326 Reclassifying Triple Negative Breast Cancers after Fluorescent In Situ Hybridization for Human Epidermal Growth Factor Receptor 2

Esther Yoon[1], *Christopher J Schwartz*[2], *Dana Warfield*[5], *Igor Deyneko*[3], *Matija Snuderl*[5], *Farbod Darvishian*[4]. [1]New York University Medical Center, New York, NY, [2]New York University Medical Center, New York, NY, [3]New York University, [4]New York University Medical Center, New York, NY

Background: Over 250,000 breast cancer cases are diagnosed annually in the US, of which 15% (n=37,500) are triple negative breast cancers (TNBC). TNBC has an aggressive clinical course with limited therapeutic options. Some studies estimate that a small proportion of TNBCs defined by immunohistochemical (IHC) stains alone, i.e. estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2)-negative, can, in fact, show HER2 amplification by fluorescent in situ hybridization (FISH) and, therefore, be reclassified as HER2-enriched. If properly classified, these patients can benefit from anti-HER2 therapy. To capture this subset of patients, we performed a now four-year-long prospective study, in which TNBCs were reflexed to HER2 by FISH. Herein, we present our findings with detailed clinicopathologic analysis of the reclassified cases.

Design: TNBC cases and ER/PR-positive, HER2-negative breast cancers from 2014 to 2017 with concurrent IHC and FISH results were analyzed. The pathology slides were reviewed by two pathologists. Stromal tumor infiltrating lymphocytes (sTILs) were defined as the percentage of all mononuclear cells within tumor stroma not in direct contact with tumor cells.

Results: Of the 253 TNBCs in the past 4 years, 13 tumors (5%) showed HER2 amplification by FISH (6 surgical excisions and 7 core biopsies). Two patients have previously identified *BRCA1* mutation. Twelve of 13 tumors (92%) were grade 3. All tumors were invasive ductal carcinoma of no special type. No apocrine morphology was noted. Notably, five tumors had ≥50% sTILs, which can be classified as lymphocyte-predominant breast cancer based on some studies. In the same time period, 41 ER/PR-positive/HER2-negative breast cancers were reflexed to HER2 by FISH, none of which showed amplification. All tumors were fixed in formalin for comparable amount of time (6-72 hours). After reclassification, approximately 40% of patients received anti-HER2 therapy.

TNBC (N=13)	Age (years)	Radiologic Size (cm)	Ischemic time (hr)	HER2 ratio	sTIL (%)	Ki-67 (%) median*
	54 (31-79)	2.4 (0.5-6.1)	1.5 (0.8-3)	3.9 (2.06-11.33)	35 (10-70)	85 (40-99)

*all values represent mean (range), unless otherwise specified.

Conclusions: Pathologists may consider reflexing HER2 evaluation to FISH in TNBCs with grade 3, high ki67 and high sTILs. If 5% of TNBCs can be reclassified as HER2-enriched tumors, based on the national statistics, annually approximately 1875 patients with TNBCs by IHC may actually benefit from anti-HER2 therapy.

327 Excision or not: Are there any clinical/histologic predictors of upgrade in papillomas?

Michael P Zaleski[1], *Yun An Chen*[2], *Daleela Dodge*[3], *Liyan Xu*[4], *Julie Mack*[5], *Alison Chetlen*, *Dipti Karamchandani*[6]. [1]Penn State Health Hershey Medical Center, Hershey, PA, [2]Penn State College of Medicine, Hershey, PA, [3]Penn State Health Medical Center, [4]Duke University, Durham, NC, [5]Penn State Health, Milton S. Hershey Medical Center, Hershey, PA, [6]Penn State Hershey Medical Center, Hershey, PA

Background: The clinical decision to excise intraductal papilloma (IDP) without atypia found on biopsy remains controversial. Conflicting data on the upgrade rates in the literature makes this decision more perplexing. We sought to look for clinical and histologic predictors (if any) which may predict upgrade in IDP.

Design: In this institutional IRB approved study, we retrospectively identified 296 biopsies (in 278 women) from 2007 to 2016 with histologic diagnosis of IDP without atypia on biopsy. Clinical, pathologic, and radiologic data for each patient was reviewed. Based on review by 2 breast radiologists, cases were placed into Incidental IDPs (no corresponding radiographic correlate), or Non-incidental IDPs (positive radiographic correlate). H&E slides of the biopsy and subsequent excision (available in 206 cases) were reviewed.

253/296(85.5%) cases were non-incidental, and 43/296(14.5%) were considered incidental. 73.1 %(185/253) non-incidental and 48.8% (21/43) incidental cases underwent excision. 12.4 %(23/ 185) non-incidental cases underwent an upgrade to cancer or high-risk lesion. This included 8 Ductal carcinoma in situ (DCIS), 8 atypical ductal hyperplasia (ADH), 6 lobular neoplasia, and 1 flat epithelial atypia. There was no histologic feature on the biopsy which predicted upgrade; however a past history of atypia was significantly associated with upgrade (Table 1). 2 of the 21 incidental cases upgraded (1 to ADH and 1 to lobular neoplasia), of note the former had a past history of ADH in the past. No incidental cases upgraded to DCIS or carcinoma. Both upgrades in the incidental group were more than 1 mm in size, and were not completely excised on the biopsy.

Table 1: Features in Non-incidental cases which upgraded vs. did not upgrade on excision

	Upgrade	Non-upgrade	P-value
Size(in mm) <1 1-2 >2	 5 (21.7%) 3 (13.0%) 15 (65.2%)	 16 (9.9%) 34 (21.0%) 112 (69.1%)	0.20*
No. of foci Multiple Single	 16 (69.6%) 7(30.4%)	 100 (61.7%) 62 (38.3%)	0.47**
Complete excision or not Completely excised Cannot tell Not completely excised	 4 (17.4%) 4 (17.4%) 15 (65.2%)	 22 (13.6%) 35 (21.6%) 105 (64.8%)	0.83*
Fragmented or not Fragmented Intact	 16(69.6%) 7(30.4%)	 103(63.6%) 59(36.4%)	0.57**
Past Medical history No medical history Past History of breast atypia/ neoplasia	 13 (56.5%) 10 (43.5%)	 125(77.2%) 37(22.8%)	0.03**

* p-values are fisher's exact tests
** p-value are Chi-square test results

These findings suggest that IDPs in patients with past history of breast atypia or neoplasia as well as non-incidental IDPs should be considered as candidates for surgical excision, given the upgrade rate of 12.4% and no definitive histologic predictors of upgrade. Incidental IDPs (if less than 1 mm and completely excised on biopsy and in a patient with no past history of breast cancer or high risk breast lesion) can be spared excision.

328 HER2/neu-Amplified Breast Cancers with HER2/CEP17 ratio ≥ 2.0 but with an Average HER2 Copy Number <4.0 Signals/Cell: An Assessment of Frequency, Immunohistochemical Correlation, and Clinicopathologic Features

Somaye Zare[1], *Leo Lin*[2], *Abrar G Alghamdi*[3], *Andres Roma*[4], *Marie Dell'Aquila*[2], *Farnaz Hasteh*[5], *Oluwole Fadare*[1]. [1]UC San Diego Health, San Diego, CA, [2]UC San Diego Health, San Diego, CA, [3]University of California, San Diego, San Diego, CA, [4]University of California San Diego, San Diego, CA, [5]Univ. of California, San Diego, San Diego, CA

Background: The 2013 ASCO/CAP updated guidelines for the interpretation of HER2/neu (HER2) amplification in breast cancers calls for the classification of cases with a fluorescence in-situ hybridization (FISH) dual probe HER2/CEP17 ratio ≥2 as *amplified,* irrespective of HER2/neu copy number. Therefore, cases with HER2/neu signals/cell <4 and HER2/CEP17 ratio ≥2 are reported as HER2/neu-positive, although there has been a lack of consensus on the validity of this approach. Here, we aim to define the frequency of this finding in breast cancers, and to comparatively assess their clinicopathologic features

Design: We retrospectively assessed reported FISH data for 1201 primary and metastatic breast cancers, all of which had been assessed for HER2/neu status using both a dual probe HER2/CEP17 FISH assay and immunohistochemistry (IHC), and all of which had been scored using 2013 ASCO/CAP criteria. We determined the frequency of HER2/neu-amplified cases with a HER2/CEP17 ratio ≥ 2.0, average HER2/neu copy number <4.0 signals/cell and average CEP17 <2.0 signals/cell (GROUP 1). These cases were compared to a second group (GROUP 2) comprised of all HER2/neu-amplified breast cancers with a HER2/neu copy number ≥4, regarding a variety of clinicopathologic features.

Results: 217 (18.1%) of 1201 cases were HER2/neu-amplified by FISH, 18 (8.3%) of which met the criteria for inclusion into group 1; the remaining 199 (91.7%) cases constituted group 2. By IHC, 11 (61.1%) of group 1 cases were negative (scores 0 and 1+), 7 (38.9%) were equivocal (score 2+) and none were positive (score 3+). IHC results in group 2 cases were as follows: 24 (12%) negative, 70 (35.2%) equivocal, and 105 (52.7%) positive (Chi square p<0.0001 for groups 1 versus 2, IHC scores 0/1+ versus 2+/3+). Overall, the 2 groups showed no statistically significant differences regarding patient age, ER and PR status, lymph node involvement, tumor grade, tumor stage, and tumor histotype distribution.

Conclusions: Cases with HER2/CEP17 ratio of ≥2.0 and a HER2/neu copy number <4.0 account for 1.5% of breast cancers and 8.3% of HER2/neu-amplified breast cancers. They were not found to be statistically distinguishable from HER2/neu-amplified cases with a HER2/neu copy number ≥4 regarding a wide variety of clinicopathologic features. However, they were frequently -61.1%- HER2/neu-negative by IHC. Accordingly, a strategy of reflexing to FISH for only IHC-equivocal cases would have missed 11 (5%) of the HER2/neu-amplified cases in this cohort.

329 Comparative Pathologic Analysis of Breast Cancers Classified as HER2/neu-Amplified by FISH using a Standard HER2/CEP17 Dual Probe and an Alternative HER2/LIS1 Dual Probe: An Analysis of 1201 Cancers.

Somaye Zare[1], *Leo Lin*[2], *Abrar G Alghamdi*[3], *Andres Roma*[4], *Farnaz Hasteh*[5], *Marie Dell'Aquila*[2], *Oluwole Fadare*[1]. [1]UC San Diego Health, San Diego, CA, [2]UC San Diego Health, San Diego, CA, [3]University of California, San Diego, San Diego, CA, [4]University of California San Diego, San Diego, CA, [5]Univ. of California, San Diego, San Diego, CA

Background: At our institution, breast cancer cases with a HER2/neu (HER2)-equivocal result by FISH using the dual probe HER2/chromosome enumeration probe [CEP17] are reflexed to a HER2/lissencephaly gene1[LIS1, 17p13.3] dual probe assay, consistent with the 2013 ASCO/CAP recommendations. However, it is unclear whether cancers that are classified as HER2-amplified with an alternate probe are clinicopathologically similar to those that are classified as such using the HER2/CEP17 probe, which may provide some insight into whether responsiveness to anti-HER2 medications is comparable between the 2 groups. Herein, we compare the 2 groups regarding their basic clinicopathologic profiles

Design: Reports for 1201 breast cancer cases were reviewed. All had initially been assessed for HER2 status using the dual HER2/CEP 17 probe. Clinicopathologic findings were compared between HER2/CEP17-equivocal cases that became HER2-amplified using the alternate HER2/LIS1 probe (group 1: n=48), primarily HER2-amplified cases using the HER2/CEP17 probe (group 2: n=169), and HER2-non amplified cases using the HER2/CEP17 probe (group 3: n=910). All cases were tested for HER2 concurrently by IHC and FISH, per institutional protocol.

Results: Of 1201 cases tested using the HER2/CEP17 dual probe, 169 (14%) were HER2-amplified, 122 (10%) were equivocal, and 910 (76%) were non-amplified. Additional testing with the LIS1 probe on the 122 equivocal cases re-classified 48 (39%) of them as HER2-amplified. Group 1 cases showed a significantly higher frequency of ER and PR positivity than group 2 cases: ER: 97.91% in group 1 versus 72.18% in group 2, p<0.0001; PR: 85.41% versus 59.17%, p=0.0009). Most group 1 cases (68%) were classified as equivocal (score 2+) by IHC whereas most group 2 cases (60%) were classified as positive (score 3+). Groups 1 and 2 showed no significant differences regarding patient age, lymph node status, tumor grade, histotype and stage distribution.

Conclusions: Reflex testing with an alternate probe reclassified 39% of breast cancers with *equivocal* HER2 results to *amplified,* and such cases comprised 22% of all HER2-amplified tumors. Among the HER2-amplified cohort, alternate probe (HER2/LIS1)-detected cases were more frequently ER and PR-positive than HER2/CEP17-detected cases, and were more frequently discordant with HER2 IHC results. These findings suggest some underlying biologic differences between these 2 groups. However, the tumors were largely comparable regarding all other clinicopathologic variables.

330 Assessing Tumor Infiltrating Lymphocytes and Genomic Alterations in Pre-Treatment Core Biopsy and Correlation with Response to Neoadjuvant Chemotherapy

Jennifer Zeng[1], *Edi Brogi*[2], *Giuseppe Floris*[3], *Gulisa Turashvili*[2], *Pedram Razavi*[1], *Jose Baselga*[1], *Rachel Sanford*[1], *Dara Ross*[4], *David Solit*[1], *Michael F Berger*[5], *Britta Weigelt*[2], *Jorge Reis-Filho*[6], *Hannah Y Wen*[2]. [1]Memorial Sloan Kettering Cancer Center, [2]Memorial Sloan Kettering Cancer Center, New York, NY, [3]University Hospitals Leuven, Leuven, [4]New York, NY, [5]MSKCC, [6]Memorial Sloan-Kettering CC, New York, NY

Background: Neoadjuvant chemotherapy (NAC) offers the benefits of downstaging the tumor in the breast and axilla, allowing for less extensive surgery and avoiding axillary dissection. Pathologic complete response (PCR) after NAC is associated with better long-term outcomes. In this study, we evaluated the correlation of tumor infiltrating lymphocytes (TILs) and genomic alterations with response to NAC.

Design: We reviewed pre-treatment breast cancer core biopsies from patients enrolled in a clinical study at our institution. All patients received standard chemotherapy, either alone, or in combination with HER2-targeted therapy for patients with HER2+ tumor. Assessment of TILs was performed by two pathologists independently following the published guidelines, and the mean score was used for analysis. ER, PR and HER2 assessment was performed following the current ASCO/CAP guidelines. PCR was defined as absence of invasive carcinoma in breast and lymph node. Pre-treatment samples with sufficient tumor content were subject to targeted massively parallel sequencing targeting up to 468 cancer genes. Statistical analysis was performed using Fisher's exact test or Chi-square test.

Results: Our study cohort comprised 112 core biopsies form 111 patients. Median age was 50 years (range 20-84). Most (101; 90%) cases were invasive ductal carcinoma no special type. Histologic grade was 2 in 32 (29%) and 3 in 80 (71%) cases. Receptor subtypes included: ER+/HER2- (53; 47%), ER-/HER2+ (14; 13%), ER+/HER2+ (15; 13%), triple negative (TN) (30; 27%). TILs counts were ≤ 10% in 77 (69%) biopsies, >10% but <50% in 22 (20%), and ≥50% in 13 (12%). The overall PCR rate was 41% (46/112). The PCR rate was 86%, 67%, 40%, and 23% for ER-/HER2+, ER+/HER2+, TN and ER+/HER2- subtypes, respectively. Higher PCR rate was significantly associated with grade 3 histology (p=0.0108), HER2+ or TN subtype (p<0.001), and increased TILs (p=0.002). Sequencing data were available for 81 pre-treatment biopsies. The most frequent somatic mutations were *TP53* (56/81; 69%), *PIK3CA* (22/81; 27%), and *GATA3* (7/81; 9%), mutated at similar frequency in PCR and non-PCR groups.

Conclusions: Tumor grade, receptor status, and increase TILs were significant predictive factors for PCR. The frequency of common mutations did not differ between PCR and non-PCR groups.

331 Tumor Infiltrating Lymphocyte Volume is a Better Predictor of Neoadjuvant Therapy Response in Triple Negative Breast Cancer

Lin Zhang[1], *Yi Tat Tong*[2], *Xiaohong Iris Wang*[3], *Songlin Zhang*[4]. [1]The University of Texas Health Science Center at Houston, Houston, TX, [2]The University of Texas Health Science Center at Houston, Houston, TX, [3]Bellaire, TX, [4]University of Texas, Houston, Houston, TX

Background: Triple-negative breast cancer (TNBC) has an aggressive clinical behavior, limited therapeutic options, and high mortality rate. Neoadjuvant therapy is a standard therapy before surgery for TNBC and patients with pathological complete response (pCR) have favorable clinical outcome. The goal of this study is to determine the morphologic features for predicting neoadjuvant therapy response in TNBC patients.

Design: Fifty-four TNBC patients who underwent breast biopsy, neoadjuvant therapy, and mastectomy in our institution during 2009-

2016 were included in the study. The H&E slides of core biopsy and surgical specimen with residual tumor were retrospectively reviewed. The pathologic parameters include histology, mitotic count, Ki67 index, stromal percentage, tumor infiltrating lymphocytes (TILs), and tumor necrosis. These pathologic parameters were compared between pCR and non-pCR group, and between primary tumor and residual tumor in the non-pCR group. Statistical analysis was performed.

Results: Among the 54 TNBC patients, 26 (48%) achieved pCR and 28 (52%) had residual cancer. There were no statistically significant differences in mitotic activity, Ki67 index, tumor necrosis, and histology between the pCR and non-pCR group. TILs were associated with pCR with statistical significance (p=0.01) and pCR group had 1.8-fold more TILs than non-pCR group. Tumor stroma percentage showed a trend in pCR group but was not significant (p=0.07). High tumor infiltrating lymphocyte volume (TILV, TILs% x tumor stroma %) was significantly associated with pCR (p=0.003). In non-pCR group, there were no significant differences in TILs, tumor stroma, TILV between the primary tumor in the core biopsy and residual tumor in the resection.

Conclusions: In this study, we confirmed the prognostic and predictive value of TILs for neoadjuvant therapy in TNBC. However, the current TILs evaluation does not include the tumor stromal percentage and may not adequately reflect the true capacity of tumor immunity. Tumor infiltrating lymphocyte volume (TILV) by incorporating the tumor stromal percentage to evaluate tumor immunity showed more predictive value than TILs alone for pCR.

332 Surgical Outcome of Benign and Atypical Vascular Lesion of Breast Diagnosed on Core Biopsy

Huina Zhang[1], *Min Han*[1], *Kavita Varma*[2], *David Dabbs*[1]. [1]Magee-Womens Hospital of UPMC, Pittsburgh, PA, [2]Magee Women Hospital, Pittsburgh, PA

Background: Benign and atypical vacular lesions of breast are uncommon findings on core needle biopsy and surgical excision has been recommended to rule out a more serious lesion. However, a recent study suggests excision may be spared for vascular lesion without atypia based on relatively low number of benign vascular lesions with follow-up excision. The aims of this study are to assess the surgical outcomes in lesions yielding benign and atypical vascular lesions on core biopsy.

Design: We retrospectively reviewed 116 patients with diagnosis of benign (108 patients) and atypical vacular lesions (8 patients) on core biopsy at our institution over a 17-year period.

Results: Among the 108 patients with benign vascular lesions, 41 patients were diagnosed with isolated vascular lesions and 67 patients were diagnosed with concomitant benign vascular lesions with other benign findings. Surgery was performed on 8 of 108 (7.4%) patients with benign vascular lesions and all of 8 (100%) patients with atypical vascular lesions. Upon excision, the majority of patients (88.8%, 16/18) retained benign final pathology with two exceptions. One patient with a diagnosis of capillary hemangioma on biopsy was upgraded to atypical hemangioma and another patient with diagnosis of atypical vascular lesion still remained the same diagnosis on excision. These two patients were followed-up clinically and radiologically for 4 and 8 years respectively and both of them are still alive without evidence of malignancy or recurrence.

Conclusions: These numbers are small, but the data supports the recent published study that benign vascular lesion may not require surgical excision. More interestingly, our results provide additional evidence that the majority of atypical vascular lesions (87.5%, 7/8) were downgraded to benign pathology on excision, and are consistent with the recent published results which showed the atypical vascular lesions on the core biopsy always follow a benign course. These findings may raise the possibility that surgery may also not warranted for the atypical vascular lesion diagnosed on core biopsy, but instead have close clinical and radiologic surveillance.

333 FOXK2 is a Novel Oncogene in Breast Cancer

Hong Zhang, New York, NY

Background: By integrative analysis of public genomic datasets of breast cancers from The Cancer Genome Atlas (TCGA), FOXK2 was found to display frequent genomic amplifications and correlated gene expression changes in breast cancer. Located in 17q25, FOXK2 gene encodes a transcriptional factor with a fork head DNA binding domain and it has not been shown to be associated with cancer-causal genetic aberrations.

Design: The status of FOXK2 was explored by mining the breast cancer TCGA datasets including 910 tumor cases and 981 normal controls. The effects of FOXK2 stable knockdown on proliferation and anchorage-independent growth in four cell lines with high FOXK2 expression (MDA-MB-231, MCF-7, HCC1954 and MDA-MB-361) using lentivirus mediated shRNAs were assessed. The oncogenic activity of FOXK2 was evaluated by colony formation assay. To identify potential interacting molecules/pathways, the RNASeq data of breast cancer cell line MDA-MB-231 with FOXK2 knockdown was analyzed.

Results: Frequent genomic amplifications of *FOXK2* were detected in breast cancers compared to normal controls in all subtypes of breast cancers classified by PAM50 from public datasets. Its overexpression was associated with poor overall survival. FOXK2 knockdown in several breast cancer cell lines inhibited cell proliferation and anchorage-independent growth. Overexpression of FOXK2 and oncogene RAS induced MCF10A cell colony formation. These indicate that FOXK2 is an oncogene in breast cancer and *FOXK2* amplification/overexpression is required for breast cancer cell proliferation. To identify its target genes, we analyzed the transcription profiling change of FOXK2-knockdown MDA-MB-231 cells compared to the control cells by RNA-seq analysis. We found 51 downregulated genes and 175 upregulated genes. With validation, CCNE2 (cyclin family), PDK1 (pyruvate dehydrogenase kinase, isozyme 1) and KDM3A (lysine (K)-specific demethylase 3A) were identified as target genes of FOXK2. The mRNA levels of all three genes were dramatically decreased after FOXK2 knockdown in MDA-MB-231, MCF7, MDA-MB-361 and HCC1954 cell lines. ChIP-qPCR results showed that transcription factor FOXK2 could bind to the promoter regions of these three genes.

Conclusions: Our data provide compelling evidence that FOXK2 is an oncogene in breast tumorigenesis and it might be a novel therapeutic target. We are assessing the incidence and significance of FOXK2 amplification in human breast cancer specimens using immunohistochemistry and FISH on TMA to determine its prognostic value.

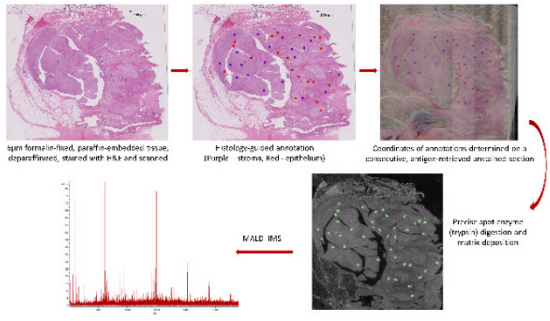
334 A Histology-Guided Matrix-Assisted Laser Desorption/Ionization Imaging Mass Spectrometry (MALDI IMS) Study of Low-Grade Phyllodes Tumor and Fibroadenoma

Lingxin Zhang[1], *Chen Yang*[1], *Audra M Judd*[2], *Nathan H Patterson*[2], *Michelle L Reyzer*[2], *Jeremy L Norris*[3], *John Pfeifer*[4], *Horacio Miguel Maluf*[1]. [1]Washington University School of Medicine, Saint Louis, MO, [2]Vanderbilt University School of Medicine, [3]Vanderbilt University School of Medicine, Nashville, TN, [4]Washington University, Saint Louis, MO

Background: The histopathologic distinction of low-grade phyllodes and certain fibroadenomas has long been a challenge due to overlapping histologic features. MALDI IMS is a powerful technique for analyzing peptides, proteins, lipids, and nucleic acid from tissue sections with spatial fidelity. We investigated the molecular signatures of low-grade phyllodes tumors and fibroadenomas (including cellular fibroadenomas) in search for differences that would allow objective separation of these lesions.

Design: Consecutive excisional biopsies of phyllodes tumors and fibroadenomas were retrieved by searching in the laboratory information system. On consensus review by three pathologists, high-grade (malignant) phyllodes tumors were excluded. One formalin-fixed, paraffin-embedded tissue block was selected for each of 9 low-grade phyllodes tumors and 10 fibroadenomas. Histology-guided annotation of the epithelial and stromal components (20 spots each) on 6µm tissue sections was followed by antigen retrieval, enzymatic digestion, and MALDI IMS analysis, to generate distinct mass spectra (**Figure**). The mass spectra from normal breast tissue stroma were acquired as an internal control. Classes of spectra were loaded into the analytical software to generate the sum of intensities from all data points at each mass to charge ratio. Differentially expressed features were identified for each component in phyllodes tumors and fibroadenomas, using the area under the receiver operator curve (AUC) of >0.8.

Results: In comparison to normal breast stroma, fibroadenoma stroma and phyllodes tumor stroma had 16 peaks with AUC>0.8 (including 3 peaks with AUC>0.9) and 36 peaks with AUC>0.8 (including 22 peaks with AUC>0.9), respectively. No differentiating features were identified between the mass spectra of phyllodes tumors and fibroadenomas in the epithelium alone, or the epithelium and stroma combined. There were 5 peaks with AUC>0.8 when comparing the stroma alone.



Conclusions: Using a histology-guided MALDI IMS approach, we identified differentially expressed features in the mass spectra between low-grade phyllodes tumors and fibroadenomas. Current data suggests more difference in the stromal component than in the epithelium. We are currently generating an algorithm to differentiate the two entities based on the data, which will be further validated on excisional and core biopsies.

335 Stromal ColX 1 is predictive of outcome in adjuvant treated ER and HER2-positive breast cancers

Chaohui Zhao[1], *Alexander S Brodsky*[2], *Dongfang Yang*[2], *Ashlee Sturtevant*[2], *Murray Resnick*[3], *Yihong Wang*[3]. [1]Brown University, Providence, RI, [2]Brown University, [3]Rhode Island Hospital, Providence, RI

Background: Factors in the tumor microenvironment including tumor infiltrating lymphocytes (TILs) and tumor associated stroma have been associated with progression and response to chemotherapies in several human malignancies including breast cancer. Previously we found that increased expression of stromal Type X collagen 1 (ColX 1) and low TILs correlated with poor pathologic response in neoadjuvant treated ER/HER2+ breast cancer. In this study, we determined whether the predictive value of ColX 1 impacted long-term outcome in patients with ER/HER2+ cancer in the adjuvant setting.

Design: ER/HER2+ breast cancer patient on adjuvant chemo- and HER2-targeted therapy from 2007-2013 were identified from the cancer registry. Patient demographic information and histological parameters such as tumor type, grade, size, lymph node status and clinical stage were recorded. ER/PR and HER2 expression were classified according to the current CAP/ASCO guidelines. TILs were analyzed as recommended by the International TILs Working Group (Salgado et al, 2015). Immunohistochemical staining of ColX was quantitated as 0, 1, 2, and 3 by intensity and percentage as previous described (Brodsky et al, 2016). The results were correlated with survival data in both univariate and multivariate analysis.

Results: Fifty-six cases with at least 5 years follow up data, advanced clinical stage (≥stage IIA, stage matching with our previous study with neoadjuvant therapy) and with available pathology material were included in this study. Mean age of the patients was 64.4 (range 34–100). Age ≥ 65 was also significantly associated with poorly long term survival (p = 0.04). Increased expression of stromal ColX 1 was strongly associated with poor long term survival in univariate and multivariate Cox proportional Hazards analysis (p <0.001; p = 0.003). The presence of TILs was associated with a trend of improved survival but not statically significant (p = 0.06). Tumor grade and stromal content were not associated with overall survival (Table 1 and Figure 1).

Table 1. Univariate Cox proportional Hazards on overall survival

Variable	HR (95% Confidence Levels)	P
Age (Above/below 65)	5.2(1.1-24)	0.04
Grade	1.3 (0.4-4.5)	0.7
ColXa1	3.87 (1.9-8)	<1e-3
TILs	0.94 (0.89-1.0)	0.06
Stroma content	0.8 (0.3-2.3)	0.7

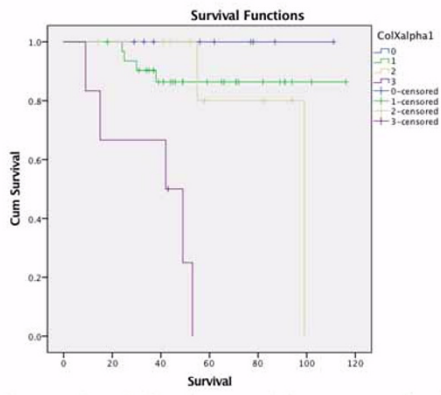


Figure 1. Kaplan Meier for ColXα1 on survival. P<0.001 on Kaplan Meier

Conclusions: ColX 1 as a predictive marker identified in the neoadjuvant setting correlates with poor long term survival in adjuvant treated ER/HER2 positive breast cancer. This study provides further support for the role of ColX 1 as a stromal factor governing heterogeneic tumor response in adjuvant setting. The evaluation of colXa1 protein levels provides a robust marker in predicting responses and warrants further evaluation in larger studies.

36 Determination of T for AJCC TNM Classification of Breast Carcinoma: Staging Implications of Utilizing Imaging Studies & Gross Examinations vis a vis Microscopic Evaluations on Needle Core Biopsies & Excisions

Elaine Zhong[1], *Timothy D'Alfonso*[2], *Katherine Simon*[1], *Alan Evans*[1], *Syed Hoda*[3]. [1]Weill Cornell Medicine, [2]Weill Cornell Medical College, New York, NY, [3]Weill Cornell, New York, NY

Background: 'T', the size of invasive breast carcinoma (ca), is an established prognostic factor. Per CAP Protocol, posted in June 2017 (www.cap.org/cancerprotocols), "the best size for AJCC T classification should use information from imaging, gross examination & microscopic evaluation". We tested the validity of this statement in a cohort of "uncomplicated" ca cases, i.e. completely excised, unifocal, previously untreated, invasive ductal ca, without extensive intraductal component, that were microscopically (micro) measurable (≤2.5 cm) on one slide.

Design: Relevant clinical & imaging records, as well as pathological material, of excised invasive ca from 32-months (2/2015-9/2017) were reviewed. Selection criteria, in addition to those listed above, included availability of imaging (magnetic resonance imaging [MRI] & either mammogram [MG] or ultrasound [US]) & needle core biopsy (NCB) data. Maximum (max) dimension on NCB was correlated with max micro size on excision, & max micro size on either excision or NCB was correlated with gross & imaging using Spearman correlations.

Results: 78 cases were reviewed. **Table 1** shows comparison of max micro extent (on either excision or NCB) & max gross extent as well as max extent on any imaging (MG, US, or MRI) modality. **Table 2** shows comparison of max micro extent on excision & NCB. **Figure 1** shows a scatterplot of gross & imaging measurements for each case in order of ascending max micro extent on excision or NCB. Spearman rank correlations showed moderate correlation between max micro extent on excision & on NCB (r=0.78, p<0.0001). Correlations with max micro extent on either excision or NCB were moderate for US (r=0.71, p<0.0001) & MRI (r=0.74, p<0.0001); weak for gross (r=0.64, p<0.0001); & nonsignificant for MG (r=0.52, p>0.01). Using gross size resulted in a decrease in at least one T stage in 16/78 (20.5%) cases, & an increase in 19/78 (24.3%). Similarly, using max imaging size resulted in a decrease in T in 8/78 (10.2%) & increase in 25/78 (32.0%). Max micro extent of invasive ca on NCB provided "true" T (greater extent than on excision) in 11/78 (7.7%) cases.

Table 1

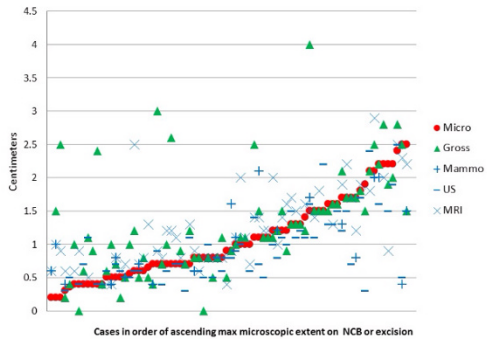
T	n	Gr>Mi	Mi>Gr	Mi=Gr	Mi>Img	Img>Mi	Mi=Img
T1a	17	11 (64.7)	4 (23.5)	2 (11.8)	1 (5.9)	13 (76.5)	3 (17.6)
T1b	27	11 (40.7)	8 (29.6)	8 (29.6)	6 (22.2)	17 (63.0)	4 (14.8)
T1c	25	5 (20.0)	5 (20.0)	15 (60.0)	7 (28.0)	16 (64.0)	2 (8.0)
T2	9	3 (33.3)	3 (33.3)	3 (33.3)	6 (66.7)	3 (33.3)	0 (0.0)
All	78	30 (38.5)	20 (25.6)	28 (35.9)	20 (25.6)	49 (62.8)	9 (11.5)

Results shown as number of cases n (%). Gr: max gross extent. Img: max extent on MG, US, or MRI. Mi: max micro extent on either excision or NCB. T: T stage based on Mi.

Table 2

T	n	Ex>NCB	NCB>Ex	Ex=NCB
T1a	17	10 (58.8)	1 (5.9)	6 (35.3)
T1b	27	20 (74.1)	3 (11.1)	4 (14.8)
T1c	25	23 (92.0)	1 (4.0)	1 (4.0)
T2	9	8 (77.8)	1 (22.2)	0 (0.0)
All	78	61 (78.2)	6 (7.7)	11 (14.1)

Results shown as n (%). Ex: max micro extent on excision. NCB: max micro extent on NCB. T: Final T stage based on max micro extent on NCB or excision.



Conclusions: Imaging studies & gross exam may be unreliable tools to determine T categorization—even in "uncomplicated" <2.5 cm invasive ductal ca. Using gross size resulted in a change in T stage in 44.8% of cases, & using max imaging size resulted in a change in T in 42.2%. Max extent of invasive ca on NCB represented true T in 7.7%.

337 Breast-Primary Solid Papillary Carcinoma Resembling Tall Cell Thyroid Papillary Carcinoma (BrTC) Shares Some, But Not All, Key Histopathological Features with Thyroid-Primary Tall Cell Variant of Papillary Carcinoma (ThTC)

Elaine Zhong[1], *Theresa Scognamiglio*[2], *Timothy D'Alfonso*[3], *Syed Hoda*[4]. [1]Weill Cornell Medicine, [2]New York Presbyterian Hosp, New York, NY, [3]Weill Cornell Medical College, New York, NY, [4]Weill Cornell, New York, NY

Background: Recent publications (*Am J Surg Pathol* 2017;41:887, *Am J Clin Pathol* 2017;147:399, *Cancer Res* 2016;76:7118) have brought attention to the histological, immunohistochemical, & molecular aspects of BrTC. However, the *histopathological* features of this "unique" & rare carcinoma (ca) *vis a vis* those of ThTC remain largely uncharacterized.

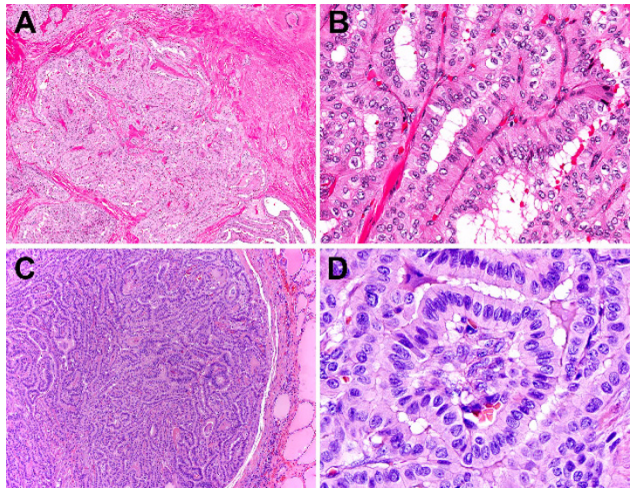
Design: Archived cases diagnosed as BrTC (all received for consultation: 2013-2017) were retrieved & reviewed. The diagnosis in each case was confirmed. Key clinical & pathological data were recorded. Comparative review of 5 randomly selected ThTC cases was performed. Key histopathological features of all BrCa & ThTC cases were assessed.

Results: Eight cases of BrTC, & 5 of ThTC, were reviewed. **Table 1** shows results thereof. BrTCs were *similar* to ThTCs in showing papillary architecture with intervening fibrous bands, tall cells (2-3x taller than wide) with nuclear grooves, & luminal ("colloid-like") secretions. BrTCs **(Fig. 1 A-B)** were *dissimilar* to ThTCs **(Fig. 1 C-D)** in showing foci of solid architecture, apical placement of nuclei (with "reverse polarity"/ "piano-keys" appearance), & absence of optically clear (true "Orphan Annie") nuclei. Luminal histiocytes were present, & tumor giant cells as well as psammoma bodies were absent, in BrTC. All BrTC patients were female, with mean age: 69.8 (range: 63-79) years. Mean size of BrTC was 1.3 (range: 0.8-1.8) cm. No case of BrTC had history of thyroid ca, & *vice versa*. All BrTC cases tested for GATA3 were (+), & all tested were (-) for TTF1 & thyroglobulin. Three of 8 (37.5%) BrTC were ER (+), 0/8 were PR (+) & 0/8 were HER2 (+). Ki67 proliferative index was <10% in 5 of 5 BrTC cases tested. Molecular studies are pending.

Table 1. Morphological Features of BrTC versus ThTC

	BrTC **(n=8)**	ThTC **(n=5)**
Architecture		
Nodular	7 (87.5)	5 (100)
Infiltrative edge	8 (100)	2 (40)
Papillae	**8 (100)**	**5 (100)**
Follicles/follicle-like	6 (75)	5 (100)
Solid	**8 (100)**	**0**
Cell Morphology		
Tall (2-3x taller than wide)	**8 (100)**	**5 (100)**
Nuclei		
Reverse polarity*	**8 (100)**	**0**
Pseudoinclusions	7 (87.5)	3 (60)
Grooves	**8 (100)**	**5 (100)**
"Orphan Annie"	**0**	**4 (80)**
Cytoplasm		
Eosinophilic	7 (87.5)	4 (80)
Vacuolated	1 (12.5)	1 (20)
Secretions		
Thin	3 (37.5)	1 (20)
Colloid/colloid-like	6 (75)	3 (60)
Scalloping	4 (50)	2 (40)
Stroma		
Fibrous bands	8 (100)	5 (100)
Calcifications		
Psammoma bodies	0	3 (60)
Calcium phosphate	2 (25)	1 (20)
Other		
Luminal histiocytes	4 (50)	0
Tumor giant cells	0	4 (80)

*: apically located nuclei.
BrTC: Breast-primary solid papillary ca resembling tall cell thyroid papillary ca, ThTC: Thyroid-primary tall cell variant of papillary ca.
Key findings are in bold.



Conclusions: Although both BrTC & ThTC show tall cells, papillary structures, nuclear grooves, & fibrous bands, the two tumors are dissimilar in other key respects: BrTC shows solid foci, "reverse polarity" of nuclei, & absence of true "Orphan Annie" nuclei. These differentiating features, among others, may preclude consideration of a thyroid primary in cases of BrTC.

FIG. 231

Molecular and Immunohistochemical Analysis of MPA

	PLAG1 FISH	**HMGA2** FISH	HMGA2 IHC	SOX10 %	CK5/6 %	ER %	p53 IHC intensity, %	Ki67 %	Pathogenic mutations	Chr gains	Chr losses
MPA1	-	-	0	95	80	0	1-2+, 2	0.5	-	-	14q22.1-q32.11
MPA2	-	-	98	100	80	3	1-2+, 30	0.5	KMT2A p.Q3203*, ARID5B p.E1039*	12q14.3	11q22.3-q24.2, 12q14.3
MPA3	-	-	0	85	100	0	1-2+, 10	2	ARID1A p.G2177fs	distal 10q, distal 20q, 22q	5, distal 6q
MPA4	+	-	0	40	95	3	1-2+, 30	2	-	-	-
MPA5	-	-	0	90	10	0	1+, 20	2			
MPA6	-	-	10	0	NA	30	1-2+, 20	0			
MPA7	-	-	0	100	NA	NA	NA	NA			
MPA8	-	-	0	5	NA	NA	NA	NA			

FIG. 257

Tumor Characteristic	All Non-BRCA Mutated Cancers (20 cancers)	ATM (2)	BLM (2)	BMPR1A (1)	BRP1 (1)	CHEK2 (4)	MUTYH (2)	PALB2 (1)	RAD50 (3)	TP53 (4)
Invasive Cancer Histotype										
Ductal	13/20 (65%)	1	2	1	0	3	1	1	1	3
Ductal w/ lobular fx	5/20 (25%)	1	0	0	1	1	1	0	1	0
Lobular	2/20 (10%)	0	0	0	0	0	0	0	1	1
Grade										
1	6/19 (32%)	1	0	0	0	3	1	*	1	0
2	9/19 (47%)	1	0	1	1	1	0		2	3
3	4/19 (21%)	0	2	0	0	0	1		0	1
Lymphocytic Response**										
Absent	7/13 (54%)	1	0	NR	NR	2	1	NR	3	0
Mild/moderate	3/13 (23%)	1	0			2	0		0	0
Brisk	3/13 (23%)	0	2			0	0		0	1
Stromal Reaction**										
Absent	2/13 (15%)	0	1	NR	NR	0	0	NR	1	0
Sclerotic	6/13 (46%)	1	0			2	0		2	1
Desmoplastic	5/13 (38%)	1	1			2	1		0	0
In Situ Component**										
Absent	4/13 (31%)	0	1	NR	NR	1	1	NR	1	0
Present	9/13 (69%)	2	1			3	0		2	1
DCIS only	6/13 (46%)	2	1			2	0		0	1
LCIS only	2/13 (15%)	0	0			1	0		1	0
Both DCIS and LCIS	1/13 (8%)	0	0			0	0		1	0

*Insufficient tissue to determine grade

**Only determined for cancers with pathology material available for review (n=13)

NR = not reviewed

FIG. 272

Table 1: Results of excisions performed for lobular neoplasia diagnosed on core needle biopsy

Diagnosis on CNB	Mean age (y)	Number of cases	Modality	LN incidental	No residual LN	Concordant	Upgrade	High risk lesions	Dis-cor-dant	Upgrade	High risk lesions
cLCIS	58	20	7 SB	4	2	7	0	1 ADH	0	-	-
			11 US	11	0	9	1 DCIS*	1 ADH 1 FEA	2	0	1 ADH
			2 MRI	2	0	1	0	-	1	0	0
pLCIS	56	2	2 SB	0	0	2	1 ILC	1 pLCIS	0	-	-
ALH	57	63	38 SB	35	16	37	0	1 FEA	1	0	0
			25 US	25	9	12	1 DCIS*	2 ADH	13	1 IDC 1 ILC	1 FEA

CNB: core needle biopsy; LN: lobular neoplasia; cLCIS: classical lobular carcinoma in situ; pLCIS: pleomorphic lobular carcinoma in situ; ALH: atypical lobular hyperplasia; SB: stereotactic biopsy; US: ultrasound-guided biopsy; MRI: magnetic resonance imaging-guided biopsy; ADH: atypical ductal hyperplasia; DCIS: ductal carcinoma in situ; FEA: flat epithelial atypia; ILC: invasive lobular carcinoma; IDC: invasive ductal carcinoma

*DCIS present away from biopsy site, not considered a true upgrade

FIG. 273

Tumor characteristics

Case No.	Histology				MYB/MYBL1 gene rearrangement					
	Review diagnosis	ACC grade	Solid component (%)	High-grade transformation	Rearrangement pattern	MYB split	MYB-NFIB fusion	MYBL1 split	MY-BL1-NFIB fusion	NFIB split
7	ACC	Low	15	No	MYB-NFIB fusion	+	+	-	-	NA
11	ACC	Low	3	No	MYB-NFIB fusion	+	+	-	-	NA
20	ACC	Low	1	No	MYB-NFIB fusion	+	+	-	-	NA
21	ACC	Low	40	No	MYB-NFIB fusion	+	+	-	-	NA
23	ACC	Low	5	No	MYB-NFIB fusion	+	+	-	-	NA
25	ACC	Low	25	No	MYBL1-NFIB fusion	-	-	+	+	NA
24	ACC	Low	0	No	MYB split only	+	-	-	-	-
12	ACC	Low	40	No	None	-	-	-	-	-
3	ACC	High	80	Yes	MYB-NFIB fusion	+	+	-	-	NA
4	ACC	High	90	No	MYB-NFIB fusion	+	+	-	-	NA
14	ACC	High	0	Yes	MYB split only	+	-	-	-	-
18	ACC	High	99	Yes	MYB split only	+	-	-	-	-
22	ACC	High	0	Yes	MYB split only	+	-	-	-	-
15	ACC	High	90	No	MYBL1 split only	-	-	+	-	-
1	ACC	High	80	Yes	None	-	-	-	-	-
17	ACC	High	5	Yes	None	-	-	-	-	-
19	ACC	High	90	No	None	-	-	-	-	-
5	IDC (cribriform)		-	-	None	-	-	-	-	-
10	IDC (cribriform)		-	-	None	-	-	-	-	-
13	IDC (cribriform)		-	-	None	-	-	-	-	-
8	IDC (basaloid)		-	-	None	-	-	-	-	-
9	IDC (basaloid)		-	-	None	-	-	-	-	-
16	IDC (basaloid)		-	-	None	-	-	-	-	-
26	IDC (basaloid)		-	-	None	-	-	-	-	-