96 Radiation Therapy Related Down-Regulation of PD-L1 on High-Grade Poorly Differentiated Sarcomas Justify the Combined Radio-Immunotherapy

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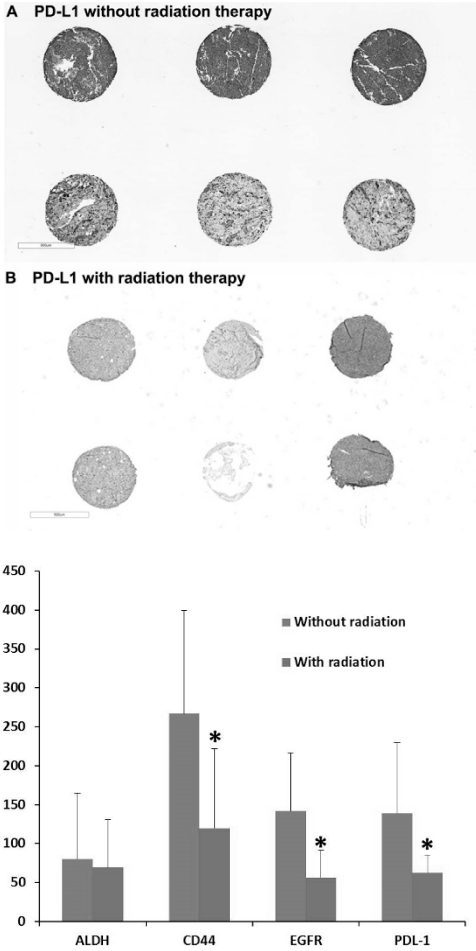
Background: Successful multimodality management of advanced soft tissue sarcomas (STS) remains a clinical challenge. Although immune checkpoint blockade has shown great promise, only a minority of patients respond. Improved biomarkers could benefit the treatment choice, since cytotoxic therapies and radiotherapy (RT) can alter the immune milieu. The objective of this study was to characterize PD-L1 expression in locally advanced STS with or without preoperative RT.

Design: Tissue microarrays (TMA) were constructed using formalin-fixed, paraffin-embedded STS cases (N=17). A composite H-scoring system was applied to analyze/quantify the protein expression. TMA sections were immunostained using a rabbit anti-human PD-L1 antibody (Sino Biological, Clone: 015). The intensity and percentage of PDL-1-positive cells were calculated and scored blindly. Patients were categorized into PD-L1 high and low-expressing based on H-score above or below the median. Parametric and non-parametric statistics were used as appropriate.

Results: Mean age was 55±21, 82% were female, and 53% of STS tumors were located on the extremity. Median tumor size was 15.5 cm (range 2.4-24.8 cm). Half of the cases received preoperative RT. We observed 9 recurrences, and 5 sarcoma deaths. Overall, PD-L1 expression was significantly lower among RT patients (62.5±23.1 vs 139±90.5, p=0.04), and tumor stem cell markers EGFR/CD44 were also significantly lower among chemotherapy patients ($p < 0.05$). Distant recurrences were more common in PDL-1 high patients (5/8, 62%) than PDL-1 low patients (2/9, 22%).



Conclusions: RT is associated with decreased PD-L1 expression in locally advanced STS, and lower PD-L1 expression is associated with improved longterm outcome. The modulation of PDL-1 expression by RT and the impact on prognosis in STS warrants further study.

97 Clinicopathological and Molecular Features of Dedifferentiated Liposarcoma with Ossification: A Comparative Study with Dedifferentiated Liposarcoma without Ossification and Extraskeletal Osteosarcoma

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Background: Dedifferentiated liposarcoma (DDLPS) sometimes exhibit heterologous differentiation, and its influence on prognostic outcome is still controversial. As for ossification, it is also not clear whether the bone component means heterologous differentiation or it can be formed by reactive (non-neoplastic) mesenchymal cells. We aimed to investigate the neoplastic nature of the bones formed in DDLPS, and make clear the clinical and pathological characteristics of DDLPS with ossification.

Design: We examined 27 cases of DDLPS with ossification by comparing them with 24 cases of DDLPS without ossification and 17 cases of primary extraskeletal osteosarcoma (ESOS) without MDM2 amplification or overexpression. The clinical and pathological findings were reviewed. Histological grade was determined using 'modified' FNCLCC grading system proposed before emphasizing the importance of tumor differentiation scoring.

Results: MDM2 amplification was confirmed in osteocytes and/or osteoblastic cells in all DDLPS cases with ossification where Fluorescence In-Situ Hybridization (FISH) was successfully performed in bone forming area (22/22). The bones found in DDLPS were mainly mature in most cases (20/27), and some of them could be described as "metaplastic." The bones were often predominantly formed in the peripheral part of the dedifferentiated area, which means close to well-differentiated liposarcoma area (14/27). Although some DDLPS cases had relatively high grade area with immature bone formation similar to conventional osteosarcoma (4/27), immature lace-like osteoid formation among highly atypical cells arranged in high density, which was often seen in ESOS (7/17), was not observed in DDLPS. Histologically, DDLPS with ossification tends to be lower grade than DDLPS without ossification, while ESOS showed highest grade (mean grade: 1.85 vs 2.10 vs 2.41). The clinical outcome, such as overall survival, of DDLPS with ossification was at least not worse than DDLPS without ossification although significant difference could not be identified.

Conclusions: The bones formed in DDLPS cases were confirmed to be neoplastic regardless of their morphology and maturity, which means osteogenic differentiation of tumor cells. We found osteogenic differentiation of DDLPS could be associated with lower histological grade, and possibly better prognosis.

98 Clinicopathological and Molecular Characterization of SMARCA4-Deficient Thoracic Sarcomas with Comparisons to Potentially Related Disease Entities

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Background: A growing number of studies have suggested critical tumor suppressor roles of the SWI/SNF chromatin remodeling complex in human cancers. The recent discovery of SMARCA4-deficient thoracic sarcomas (SMARCA4-DTSs, Le Loarer et al. Nat Genet, 2015) has added to the list of tumor groups with the *SMARCA4* inactivating mutation.

Design: To better characterize these tumors and establish their nosological status, we undertook a clinicopathologic and molecular analysis of 12 SMARCA4-DTSs and compared them with malignant rhabdoid tumors (MRTs), epithelioid sarcomas (ESs), and SMARCA4-deficient lung carcinomas (SMARCA4-DLCs).

Results: Eleven men and one woman with SMARCA4-DTS (aged 27-82 years, median 39 years) were included in the study. Most of the patients had heavy smoking exposure and pulmonary emphysema/bullae. The primary tumors were large and involved thoracic region in all cases, and simultaneously affected abdominal cavities in 3 cases. The patients followed a rapid course, with a median survival of 7 months. Histologically, all tumors showed diffuse sheets of mildly dyscohesive, relatively monotonous, undifferentiated epithelioid cells with prominent nucleoli. Immunohistochemically, all tumors demonstrated a complete absence (8 cases) or diffuse severe reduction (4 cases) of SMARCA4 expression. Cytokeratin, CD34, SOX2, SALL4, and p53 were expressed in 50%, 83%, 83%, 83%, and 70% of cases, respectively. SMARCA2 expression was deficient in 92% of cases. The targeted sequencing was successful in 5 cases and demonstrated the inactivating *SMARCA4* mutation in each case (including that with reduced SMARCA4 expression), and additionally uncovered alterations in *TP53* (5/5), *NF1* (2/5), *CDKN2A* (2/5), *KRAS* (1/5), and *KEAP1* (1/5), among others. In a comparative analysis, aside from clinical and some morphological differences, immunohistochemical profiles were found different among entities: 13 MRTs expressed CD34 in 46%, SOX2 in 77%, SALL4 in 92%, and p53 in 0% of cases, while they lacked SMARCB1 in 85%, SMARCA4 in 15%, and SMARCA2 in 50% of cases; 15 ESs expressed CD34 in 73%, SOX2 in 7%, and SALL4 in 7% of cases, while they lacked SMARCB1 in 100% and SMARCA2 in 13% of cases; 12 SMARCA4-DLCs expressed CD34 in 0%, SOX2 in 33%, and SALL4 in 0% of cases, while they lacked SMARCA4 in 100% and SMARCA2 in 8% of cases.

Conclusions: SMARCA4-deficient thoracic sarcomas constitute a unique entity that requires recognition and differentiation from other epithelioid malignancies in adults.

Breast Pathology

99 Expression of Met and Androgen Receptors in ER Negative Breast Cancer

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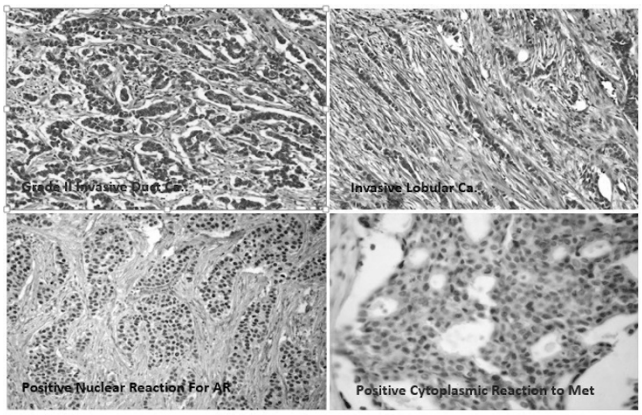
Background: Human breast cancers are heterogeneous. This heterogeneity may originate due to differences in the target cell population and/or it may be the result of different combinations of mutations in a normal breast progenitor cell. New therapeutic targets are needed in breast cancer, particularly in patients with TNBC and the related basal-like subgroup. The Met tyrosine kinase receptor activates cell proliferation and a strong relationship between high HGF/Met signaling and tumor progression was found. The biologic roles of Androgen receptors in the breast are incompletely understood since it is unclear whether the effects of androgens on breast cells are predominantly proliferative or anti-proliferative. The purpose of this study is to determine the prognostic value of Met and AR expression in ER negative breast cancer patients that might be useful information to treat breast cancer especially TNBC.

Design: Histologic sections from 60 cases of ER negative cancer breast were evaluated using immunohistochemistry with Androgen (AR 441 Thermo Fisher, Ref No.443-R7) and Met (Novusbio NBP2-44306SS) then evaluated compared to PR, HER-2 using a standard avidin–biotin–peroxidase system.
Results: Out of the 60 breast cancers, 90% are positive for AR and 86% are positive for Met.



There was a significant positive correlation between *AR intensity* with tumor type, multicentricity, DCIS and Her 2 ($P<0.05$). Met intensity scores were significantly correlated with patient's nodal stage, DCIS and HER 2 ($P<0.05$). A significant positive association was observed between the multicentricity, nuclear grade, lymphovascular invasion, PR and HER 2 ($P<0.05$) and AR expression *percentage*. On the other side the Met expression *percentage* has shown significant association with multicentricity and Her 2 expression ($P<0.05$).
Conclusions: There is a significant correlation of Met and AR intensity with the clinicopathological prognostic parameters. The levels of AR and Met expression were relatively high as most studies stated. The activation of Met signaling pathway plays an important role in tumour-genesis of breast cancer and the patients might benefit from drug therapy targeting Met in cases showing expression of such receptor.

100 Composite Analysis of Immunological and Metabolic Markers Defines Novel Subtypes of Triple Negative Breast Cancer (TNBC): Prognostic Significance and Implications for Immunotherapy

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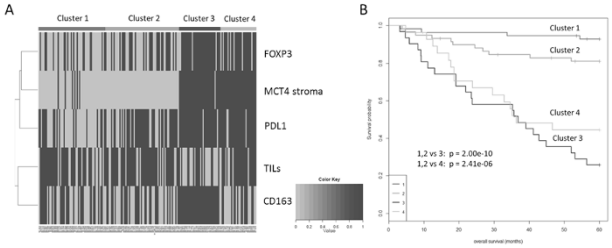
Background: Tumor biology is influenced by the metabolic and immunological environment. The development of clinically effective immune checkpoint inhibitors provides an opportunity to augment the intrinsic antitumor immune response. Similarly, targeting altered tumor metabolism is a new promising therapeutic strategy. We investigated the inter-relationship of immune response regulators (PD-L1, FOXP3, and CD163), glycolytic metabolism (MCT4), and their association with survival in TNBC.
Design: Tissue microarrays (TMAs) were prepared from 183 largely consecutive patients with TNBC diagnosed between 1995 to 2005. Clinicopathologic data including survival were compiled. The TMAs were stained with the following antibodies: PD-L1 (Cell Signaling, dilution 1:600), CD163 (Cell Marque, prediluted), FOXP3 (Abcam, dilution 1:200), and the glycolytic marker MCT4 (Santa Cruz, dilution 1:250). Expression of IHC markers was categorized semi-quantitatively using published criteria. Stromal tumor infiltrating lymphocytes (TILs) were assessed based on criteria developed by Denkert and colleagues. Correlation analysis, K-means clustering, and Kaplan-Meier survival analysis were performed in R.
Results: In univariate analysis, elevated levels of PD-L1, CD163, FOXP3, and stromal MCT4 staining were associated with significantly decreased overall survival. In contrast, high levels of TILs were associated with improved survival. K-means clustering of the markers defined four distinct TNBC subtypes. The two clusters harboring TILs, but low levels of immune-suppressive markers had overall good prognosis. In contrast, the two populations positive for PD-L1, FOXP3, and stromal MCT4 showed poor survival. Interestingly one subtype had high TILs with minimal CD163 expression, suggesting that immune checkpoint therapy could be effective in this subtype. The other PD-L1, FOXP3, MCT4 high cluster was enriched for CD163 macrophages with minimal TILs; therapies targeting M2 macrophages or glycolytic environment could be effective in this subtype.
Conclusions: Immune and metabolic markers stratify TNBC into subtypes that have prognostic significance and implications for therapies targeting immune checkpoints and tumor metabolism.



A. K-means clustering analysis plot. Cluster 3 and 4 both show positive staining for FOXP3, PDL1, and stromal MCT4. They differ in CD163 staining and TILs. B. Kaplan-Meier plot indicating survival probability based on expression clusters. Note decreased survival in cluster 3 and 4.

101 Patients with Breast Cancer and Non-*BRCA* Mutations: A Clinicopathologic Correlation

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Background: Genetic mutations that confer an increased risk of breast cancer (BC) have been the subject of much research, most famously *BRCA1* and *BRCA2*. As genetic testing tools have evolved and become more widely available, other mutations have started to come to the forefront. The tendency of *BRCA1* patients to develop triple negative high grade invasive ductal carcinomas, often with an inflammatory infiltrate is well documented, however less is known about the histology found in patients with other high risk mutations. In this study, we reviewed the BC slides from patients with a non-*BRCA* high risk mutation to determine if a certain histologic type or tumor marker status (ER, PR and HER2) is associated with any specific mutation.
Design: After IRB approval, 27 women with mutations in *CHEK2*, *PALB2, ATM, BRIP1* and/or *BARD1* genes and a personal history of invasive cancer (IC), DCIS or LCIS were identified. Slides were available for review for 22 patients. Age at diagnosis and ER/PR/HER2 results were obtained from the EMR. IC that is ER/PR positive and HER2 negative was classified as luminal. Any HER2 positive IC was classified as HER2 and ER/PR/HER2 negative IC was classified as triple negative (TN).
Results: The average age at diagnosis did not vary widely between the groups, however, 3/7 (43%) women with PALB2 and 2/15 (13%) with *CHEK2* were diagnosed at ≤40yo. 7 women have CIS only (26%) and all are in the *CHEK2* group (5 DCIS and 2 LCIS). The remaining 20 women developed 27 distinct ICs (see Table). Bilateral BC was diagnosed in 2/7 *PALB2* (29%), 1/15 *CHEK2* (7%), 1/2 *ATM* (50%) and 1/2 *BRIP1* (50%). 21/27 (78%) of the ICs are ductal and 6 (22%) are lobular; no other special types are identified. ER, PR and HER2 results are available on 25/27 (93%) ICs. Only 1 patient is TN. The 3 HER2 ICs are also ER/PR positive (triple positive).

Mutation	N (patients)	N (ICs)	Av. Age	ILC	Lum	HER2	TN
CHEK2	15	9	51	3 (33%)	9 (100%)	0	0
PALB2	7	11	56	2 (18%)	10 (91%)	0	1 (9%)
ATM	2	3	52	0	2 (67%)	1 (33%)	0
BRIP1	2	3	53	1 (33%)	2 (67%)	1 (33%)	0
BARD1	1	1	57	0	0	1 (100%)	0

Conclusions: In our patient population, *CHEK2* was the most common non-*BRCA* mutation identified (15/27; 55%) followed by *PALB2*. All the women diagnosed with BC at ≤40yo had one of these two mutations. ILC was seen most often in association with *CHEK2*. In contrast to *BRCA1*, 96% of the ICs were hormone receptor positive, with the vast majority being luminal-type ICs. The 3 HER2 ICs were triple positive.

102 PITX2 Expression Is Elevated in a Progressive Manner in Breast Papilloma and Triple-Negative Breast Carcinoma

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Background: Paired-like Homeobox Transcription factor-2 (*PITX2*) is a direct downstream target of WNT/β-Catenin pathway mediating cell proliferation during development and may be associated with tumor progression. Recent studies have identified this molecule as being associated with early recurrence in breast cancer, but it has not been well characterized. In this study, we optimized an antibody for evaluation of breast tumors and report on the differential expression of PITX2 in papilloma and invasive triple negative breast carcinomas (TNBC) with recurrence.
Design: In this study, we detected PITX2 in tissue microarrays of three breast tissue groups: control group (no significant histopathology), papilloma with and without atypia group and TNBC group. Histopathology of each specimen was reviewed by at least two pathologists in a double-blind fashion. Smooth muscle actin (SMA) was used as internal control. ImageJ (NIH) was used for cell counting (> 1,000 cells were counted per specimen). The mean of percentage of PITX2 positive cells and standard deviation (SD) were calculated (mean ± SD). $p<0.05$ is considered significant.
Results: PITX2 is expressed in an extremely low level in the control group (0.32% ± 0.16%), moderately high in the papilloma group (14.62% ± 2.71%) and highest in the TNBC group (29.48% ± 8.79%).

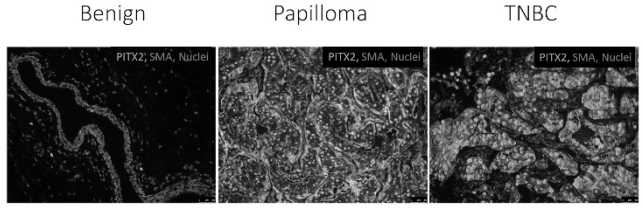

Figure 1. Fluorescence staining of PITX2 (green), smooth muscle actin (red) and nuclei (blue) in benign group, papilloma group and TNBC group.

The expression levels of PITX2 in the control group was significantly lower than that in the papilloma group (p=0.007) and TNBC group (p=0.03).

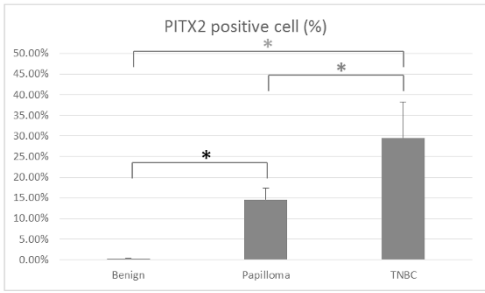

Figure 2. The percentage of PITX2 positive cells in benign group, papilloma group and TNBC group, (mean ± SD). (* p<0.01, * p<0.05,* p>0.05)

SMA staining patterns are consistent with the histopathology.

Conclusions: PITX2 level is negative in the control group, and elevated in a progressive manner in the benign tumor group and TNBC group, suggesting it may play a role in tumor initiation, promotion and/or progression. More Wnt signaling components, such as β-catenin, Wnt1 and Dvl will be tested in the future.

103 Not All Ductal Carcinoma In Situ (DCIS) Are Created IDLE (Indolent Lesions of Epithelial Origin)

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Background: Mammographic screening has increased the incidence of DCIS, but this has not been accompanied by a decline in the incidence of invasive carcinoma (IC). Consequently, the surgical treatment of DCIS has recently been questioned, with some advocating only surveillance (+/- neo adjuvant endocrine therapy) after a core biopsy (cbx) diagnosis of DCIS. This prompted us to examine the predictive value of a cbx diagnosis of DCIS, particularly the upgrade rate to IC, and identify associated factors.

Design: Using the pathology database, we identified 2943 cases of DCIS diagnosed on cbx from 2000 to 2015, of which 229 were upstaged to IC (8%). Clinical and pathologic features were studied.

Results: The age of the patients ranged from 25 to 90 (avge=59 yrs). DCIS presented with calcifications in 66% (widespread in 17%), a mass/density in 30% (heterogeneous in 63%) and MRI enhancement in 4%. DCIS grades were as follows: low=30(13%), intermediate=83(36%) and high = 116(51%). Necrosis was present in 152 cases (66.4%), comedo type in 99(43%). The T stage of the IC was as follows: microIC = 36(16%), T1a=118(52%), T1b=35(15%), T1c=28(12%), T2=9(4%) and T3=3(1%). The IC were ER+ in 80% and Her2+ in 16%. On the excision specimens, DCIS was extensive in 120 cases (52%), seen predominantly in the microIC and pT1a IC (66%) cases. 167 patients underwent axillary lymph node (ALN) sampling which staged as follows: N0=141(85%), N0(i+)=14(8%) and N1=12(7%).The N1 were further subclassified by T stage as follows: T1a=1, T1b=4, T1c=2, T2=4, T3=1.

Conclusions: The upstaged IC were predominantly (95%) <2cm, more than 2/3rds of which were <0.5cm, most of which were accompanied by extensive DCIS. Approximately half the upgrades were associated with high grade DCIS, especially with comedo necrosis; nevertheless, the other half due to low and intermediate grades DCIS, should not be underestimated. ALN positivity was low but occurred in 3% of the carcinomas <2cm. Our findings indicate that one must be cautious when treating cbx diagnosed DCIS non surgically with surveillance (+/- neo adjuvant endocrine therapy), given the upstage to IC (8%) and ALN metastases (7%). This was mostly due to sampling error in identifying a small IC in the setting of diffuse heterogeneous or multifocal lesions/calcifications. These findings have therapy changing implications from adding ALN sampling to chemotherapy, including anti Her2 agents.

104 PD-L1 *Expression in Male Breast Carcinoma and Its Correlation with Patient Demographics, Histologic Grade and Prognostic/Predictive Markers.*

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Background: Programmed death-ligand 1 (PD-L1) expression in cancer is associated with increased aggressiveness and poor prognosis, and is a novel target for cancer therapy. The expression profile of PD-L1 in male breast cancer is not widely known. In this study, we evaluated its expression in male breast cancer, and correlated it with patient demographics and tumor characteristics.

Design: 23 male breast cancer cases (6 invasive ductal carcinoma [IDC], 5 DCIS, 8 IDC with DCIS, 1 pleomorphic lobular carcinoma, 1 solid papillary carcinoma, and 2 metastatic carcinoma) were selected for RNA in situ hybridization (ISH) using a novel multiplex nucleic acid method (RNAscope®, Leica BioSystems, Buffalo Grove, IL) with probe Hs-CD274 (Advanced Cell Diagnostics, Newark, CA) and the Bond III autostainer (Leica Biosystems). With appropriate controls, PD-L1 expression was documented along with the percentage positivity.

Results: Twelve of twenty-three cases (6 of 6 IDC [100%], 2 of 5 DCIS [40%], 3 of 8 IDC with DCIS [38%], and 1 of 1 pleomorphic lobular carcinoma [100%]) had positive PD-L1 expression by RNA ISH (3+ intensity, percent positivity range 1% to 20%). The distribution of PD-L1 expression with regards to patient demographics, histologic grade, and prognostic/predictive markers are listed in table 1. Of note, 100% of cases with grade 3 IDC or pleomorphic lobular carcinoma, and 75% of cases with intermediate/high Ki-67 had PD-L1 expression.

Factors		PD-L1 status		
		n	Positive	Negative
Age (years)	< 50	7	3 (43%)	4
	> 50	16	9(56%)	7
Race	Caucasian	8	3 (38%)	5
	African American	12	6 (50%)	6
DCIS-Grade	Low/intermediate	10	3 (30%)	7
	High	3	2 (67%)	1
IDC	Grade 1/Grade 2	10	5 (50%)	5
	Grade 3	4	4 (100%)	0
	Metastatic	2	0 (0%)	2
Hormone Status	ER+/PR+	21	12 (57%)	9
	ER+/PR-	1	0 (0%)	1
Her2	Positive	5	4 (80%)	1
	Negative	12	6 (50%)	6
Ki-67	Low	1	0 (0%)	1
	Intermediate/high	12	9 (75%)	3

Conclusions: Breast cancer is a relatively rare cancer in men with fewer therapeutic options. Our study demonstrates that more than half of IDC in men (9/14, 62%) have PD-L1 overexpression. This provides an early insight into the possible therapeutic utilization of anti-PD-L1 therapy in male breast cancer patients, especially in those tumors with histology grade 3, and with intermediate/high proliferative index.

105 Underestimationof Ductal Carcinoma In Situ at Core Needle Biopsy

Sophia Apple. City of Hope, Duarte, CA.

Background: The original goal of early detection of breast cancer by screening mammogram yielded an increase in incidence of ductal carcinoma in situ (DCIS) from 3% to 20-25%. However, the removal of DCIS has not been accompanied by a reduction in the incidence of invasive breast cancer and aggressive treatment of all DCIS has not led to reduction in breast cancer mortality. "One size fits all" for treatment of DCIS concept is now being challenged by current growing literature which suggest that majority of DCIS detected by screening mammogram should be considered as a "risk factor" for invasive carcinoma, low to even intermediate grade DCIS diagnosed on core needle biopsy (CNB) does not need to be a target for screening and early detection, treatment option may be given to patient for observation without excision if DCIS is not high grade, and radiation therapy should not be routinely offered after lumpectomy for DCIS. We questioned how many DCIS diagnoses in CNB, and how many low to intermediate grade DCIS had underestimation of invasive carcinoma on excision.

Design: This is a single-institutional study spanning 13.5 years composed of 5,750 CNB samples from 2003-2015. We evaluated clinical, imaging findings, BIRADS, age, size of needle, grade of DCIS on pathology report and upgrade rate of invasive carcinoma on excisional biopsy.

Results: A total of 855 cases (14.9%) had a diagnosis of DCIS on the CNB. Excision was done on 752 patients (88%) and 103 patients (22%) were lost to follow-up. Of those who had excision, 173 (23%) had invasive carcinoma, 505 (67%) had DCIS and 71 (10%) had wither ADH or benign lesions on excision. A total of 100, 369 and 386 patients had a diagnosis of DCIS grade 1, 2 and 3 on CNB respectively. From Grade 1, 2 and 3 DCIS on CNB, 22 (22%), 70 (19%) and 81 (21%) had underestimation of IDC on excision respectively.

Conclusions: Upgrade rate of invasive carcinoma from DCIS on the CNB is 23% from our study. Combining grade 1 and 2 DCIS on the CNB were upgraded to invasive carcinoma is 114/439 (26%). Upgrade to IDC diagnosed on excision was more likely to be high grade DCIS, mass lesion on imaging and age less than 50 but no significant differences were seen for BIRADS and larger size of needle used for core biopsy. Our study supports excisional biopsy in all DCIS diagnosis on CNB regardless of grades.

106 Incidence and Significance of Fluorescent 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. City of Hope, Duarte, CA; UCLA, Los Angeles, CA.

Background: The most recent ASCO-CAP guidelines redefined *HER2* gene amplification back to the original FDA value with the Her2/CEP17 ratio of ≥ 2.0 in 2013. Also, the new criteria were added stating that the *HER2* copy numbers of ≥ 6.0 to be positive for amplification even if the ratio is < 2.0. We retrospectively evaluated Her2/CEP17 ratio in relationship with *HER2* copy numbers to find out the incidence and significance of the HER2 copy numbers of ≥ 6.0 and whether our clinicians are treating the patient with anti-HER2 therapy.

Design: A total of 1404 cases were included in the study from 2011-2015. Evaluations of Her2/CEP17 ratio, *HER2* copy number, IHC for *HER2* and clinical data were obtained. Polysomy 17 was verified by using another probe RARA probe on 17q21 which is a proxy for 17q and is outside the c17 centromere region.

Results: From 1404 samples, FISH amplified cases with ratio of ≥ 2.0 were found in 237 (17%) cases, all of which had *HER2* copy number of ≥ 6.0. From 1160 (83%) cases of non-amplified cases, only 25 (2.1%) cases from 22 patients had *HER2* copy number of ≥ 6.0, all of which had polysomy 17 with ≥ 3.0 CEP17 signals per nucleus. Overall incidence of HER2 copy number of ≥ 6.0 without Her2/CEP17 ratio of ≥ 2.0 incidence was 1.8%. Of 25 cases with *HER2* copy number of ≥ 6.0, only one case had IHC score of 3+, 9 (36%) had 2+ and 15 (60%) had either 0 or 1+. *HER2* copy

number of ≥ 6.0 without ratio of ≥ 2.0 cases are commonly seen with neoadjuvant chemotherapy setting; 9 of 22 (41%) cases in our study. 7 of 22 (32%) patients had lymph node metastasis. 13 (59%), 4 (18%), 44 (18%), and 1(5%) patients had luminal A, luminal B, triple negative and Her2 positive subtypes respectively. 11 of 22 (50%) patients received anti-HER2 therapy. Four patients had metastatic disease and within this group, three had died of breast cancer.

Conclusions: The incidence of *HER2* copy numbers of ≥ 6.0 in the setting of Her2/CEP17 ratio of <2.0 is rare, 1.8% in our study, all of which showed polysomy 17. 41% of the cases were seen after neoadjuvant chemotherapy. Polysomy 17 cases are more commonly associated with equivocal IHC score of 2+ result. Our oncologists are not uniformly treating with anti-HER2 therapy in the setting of *HER2* copy numbers of ≥ 6.0 found even in the core needle biopsy.

107 Androgen Receptor Positive Triple Negative Breast Cancer (TNBC): Clinicopathologic and Prognostic Features

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Background: TNBC is a highly heterogeneous disease with aggressive behavior and poor overall survival. Treatment has been challenging due to its heterogeneity and lack of predictive markers. Gene expression analyses have recently stratified TNBC into six molecular subsets, one of which is characterized by androgen receptor (AR) overexpression. Preliminary studies demonstrate that AR expressing TNBC may benefit from targeted antiandrogen therapy. This study determines the clinicopathologic features of AR expressing TNBC and the utility of AR as a prognostic/predictive marker.

Design: 137 consecutive invasive TNBCs, initially treated by surgery from 2008 to 2012 were selected. Data including patients' age, tumor size, grade, lymph node(LN) status, proliferation rate and follow up were collected. Carcinomas were fixed and evaluated according to the 2007 CAP guidelines. Representative sections were immunostained with AR and evaluated by two pathologists as the percentage of cells exhibiting nuclear staining. Concordant results were recorded. 25% cutoff value (estimated by time-dependent ROC curves) was used for AR. Clinicopathologic features and outcome of AR+ and AR- TNBC were compared using Cox analysis, Kaplan-Meier curves and log-rank tests.

Results: 25% cases expressed AR. AR+TNBC occured in women varying in age from 40 to 89 (65+/-13). Tumor size varied from 0.2 to 7.7 cm (2.4+/-1.5 cm). 82% were high grade and 50% showed axillary LN metastasis. Disease free survival (DFS) varied from 1.2 to 34.1 (median 13.4) months.

35% were apocrine type.

AR+TNBC occured in older women, demonstrated the apocrine phenotype more frequently and showed a lower proliferation rate than the AR-TNBC (p<0.001). No difference was noted in DFS.

Tumor size (p=0.049 (0.975-1.025), LN status (p=0.048 (0.217-3.677) and stage (p=0.022 (0.184-3.446) of the AR+ cases correlated with DFS.

Variables	Total (n=137)	% AR+ (n=34)	% AR- (n=103)	P-value
Age				<<0.001
<50	43	18	36	
50-70	65	44	48.5	
>70	29	38	15.5	
Apocrine Differentiation				<0.001
Present	17	35	5	
Absent	118	65	90	
Ki-67(%)				<<0.001
≤10	6	9	3	
11-21	14	23	6	
>21	117	68	91	

Conclusions: AR+TNBC comprise 25% of all TNBC, occur in older individuals, have a apocrine phenotype in 35% and a low proliferation rate.

TUmor size, LN status and stage are prognostic.

108 Apocrine Carcinomas Are a Distinct Subset of Androgen Receptor Positive (AR+) Triple Negative Breast Carcinomas (TNBC)

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Background: Gene expression analysis have recently demonstrated AR expressing TNBC as a distinct subset of TNBC that occurs in older women and may show a less aggressive course raising the possibility of AR as a predictive marker for androgen antagonists. Apocrine carcinomas (ApoCA+) are a rare subtype of ductal carcinomas known to express AR. This study evaluates the clinicopathologic features of ApoCA+ in the context of AR+TNBC in order to determine its utility as a predictive phenotype.

Design: From a series of 135 consecutive invasive TNBCs, treated initially by surgery and diagnosed from 2008 to 2012, 34 AR+ cases were retrieved from our database. Information including patients' age, tumor size, grade, lymph node (LN) status and results of Ki67, EGFR, CK5/6 and p53 immunostains were recorded. Follow up ranged from 1.2-34.1 (mean 13.2, median 11.5) months. H&E sections of the 135 TNBC were reviewed to select ApoCA+, diagnosed by the presence of at least 70% of the tumor cells showing apocrine morphology. Clinicopathologic features including disease-free survival (DFS) of the AR+ tumors with and without apocrine morphology were compared statistically using Cox analysis, Kaplan-Meier curves and log-rank tests.

Results: Of the 34 AR+ cases, 12 were ApoCA+, and 22 ApoCA-. An additional 5 ApoCA+ cases were identified in the AR- group.

ApoCA+ showed lower tumor grade and proliferation rate, but increased LN metastasis (p</=0.5) than ApoCA-. No difference was noted in DFS between the two groups.

Variables	AR+ ApoCA+, n=12	AR+ ApoCA-, n=22	p-value
MBR			0.012
I	0	1	
II	5	0	
III	7	21	
LN			0.011
pN0	3	14	
pN1(1-3)	7	3	
pN2(4-9)	2	1	
pN3(≥10)	0	0	
Ki-67,%			<<0.001
≤10	3	0	
11-21	6	2	
>21	3	20	
EGFR,%			0.011
≤15	2	4	
>15	10	18	

Conclusions: Apocrine carcinomas

1. comprised 13% of all TNBC and 71% of AR+TNBC; 29% were AR-.
2. form a distinct subgroup of AR+TNBC with low tumor grade and proliferation rate but increased frquency of axillary LN metastasis as compared to AR+TNBC lacking apocrine phenotype.

109 RespondR Signature Identifies Triple-Negative Breast Cancer Patients Who Are Likely to Be Insensitive to Taxane Treatment

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Background: A significant number of triple-negative breast cancer (TNBC) patients achieve pathological complete response (pCR) and improved relapse free survival from neoadjuvant anthracycline-taxane (AT) based chemotherapy. However, the 3-year distant metastasis free survival (DMFS) probability for AT-treated TNBC patients is only about 0.50. A test is needed that identifies the AT-insensitive patients who are candidates for an alternative therapy

Design: Gene expression measurements of 296 chemotherapy-treated TNBC patients were divided into training and validation sets balanced for clinical traits and pCR rate. An innovative multistate gene methodology was used to identify predictors of pCR in the training set and confirmed in the validation set, along with higher DMFS in the predicted AT-sensitive group. The RespondR score was calculated for TNBC patients in The Cancer Genome Atlas (TCGA) treated with a taxane (n = 84), and TNBC patients in GSE18864 with neoadjuvant cisplatin treatment (n = 24).

Results: Analysis of the training set (n = 118; 34 pCR) identified 19 genes that were most predictive of pCR. RespondR Score was developed using these genes to continuously stratify patients by probability of pCR. In the validation set (n= 178; 60 pCR), RespondR stratifies patients into discrete groups of low (RR-low, 38%), moderate (RR-moderate, 18%) and high (RR-high, 44%) sensitivity with rate of pCR 0.20 (RR-low), 0.19 (RR-moderate), 0.55 (RR-high) and 5-year DMFS probability 0.39 (RR-low), 0.66 (RR-moderate) and 0.75 (RR-high). In the RR-high group mitosis-related genes are up-regulated and genes involved in transmembrane transport and ion transport are down-regulated. Taxane-treated TNBC patients in TCGA with high RespondR score (46%) have significantly (p = 0.046) better 5-year relapse-free survival (0.92, 95%CI 0.82-1.0) than patients with low RespondR score (0.68, 95%CI 0.52-0.89). In the TCGA samples with low RespondR score, loss of the gene PTEN is significantly (p = 0.03) associated with relapse. In the dataset GSE18864, 50% of the patients with low RespondR score achieve partial or complete pathological response to cisplatin treatment

Conclusions: The RespondR Score accurately predicts those TNBC patients likely to achieve pCR and improved survival using AT neoadjuvant chemotherapy. RespondR can provide critical information to patients and physicians deciding between AT and an alternative therapy.

110 Granulomatous Mastitis: A Two-Institution Ten-Year Retrospective Review

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Background: Granulomatous mastitis is an uncommon inflammatory process which may signify infection or manifestation of a systemic disorder; excluding these etiologies, a diagnosis of Granulomatous Lobular Mastitis (GLM) may be rendered. A subset of GLM referred to as Cystic Neutrophilic Granulomatous Mastitis (CNGM) is characterized by suppurative granulomas with cystic spaces variably lined by neutrophils. CNGM is associated with corynebacteria and bacteria may be noted in a subset of the cysts. The purpose of this study was to review GLM with the potential to reclassify a subset as CNGM.

Design: Pathology databases from two institutions in distinct urban areas were searched for granulomatous mastitis; corresponding H&Es and special stains were reviewed to confirm granulomatous mastitis. Associated microbiology and patient (pt) data was collected. Inflammation due to prior surgical site was excluded; lesions were required to be within breast parenchyma. The minimum criteria for categorizing as CNGM was the presence of granulomatous inflammation (typically suppurative) with cystic spaces variably lined by neutrophils; though bacteria on H&E and/or tissue gram stain (H&E/gram) was noted, neither histologic nor culture evidence of bacteria was required.

Results: 43 pts with granulomatous mastitis were identified: all were female, a wide age range (19-76yo) was noted. 67% met criteria for CNGM. Typically scant, 48% of CNGMs had bacteria on H&E/gram. Of 21 CNGMs with bacterial culture, 76% were positive. Whereas 69% of CNGMs with positive bacterial culture demonstrated corynebacteria, none of the non-CNGMs did. 14 cases did not meet criteria for CNGM; of these, 2/14 had bacteria on H&E/gram and 3/5 cases had positive bacterial culture. 5 pts (19-37yo) had nipple piercings: 4 were African-American (AfAm), 4 had bacteria on H&E, 4 were CNGM, all (4/4) with bacterial culture were positive (one with corynebacteria). CNGMs tended to be younger (avg 36yo; range 19-50yo) than non-CNGMs (avg 47yo; range 23-76yo). Of CNGM, 28% were AfAm, 21% Caucasian (C), 24% Asian (As), 21% Hispanic (His), 6% NA; of non-CNGM, 57% AfAm, 22% C, 14% His, and 7% As.
Conclusions: GLM is often characterized by a protracted clinical course and tendency to recur. As lack of response to antibiotics is common, steroids are frequently used to treat GLM. As a histologic subtype of GLM, the data presented here support CNGM as an infectious disease and highlight nipple piercing as a potential risk factor. Given an association with bacteria including corynebacteria, the recognition of CNGM as an infectious process is crucial in guiding pt management.

111 Breast Cancers with < 4 HER2 Signals/cell and Amplified HER2:CEP17 Ratios by FISH: Pathologic Features and Clinical Outcomes of Cases with "CEP17 Monosomy"

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Background: When using dual probe HER2 FISH, breast cancers with very low (< 4) mean HER2 signals/cell can be technically considered HER2 amplified by ratio if the mean CEP17 control signals are reduced and the HER2:CEP17 ratio is ≥ 2.0 (referred to here as "CEP17 monosomy"). This is a controversial subcategory of HER amplification with limited information on clinical-pathologic features and response to treatment.
Design: Breast cancers with HER2 FISH results from 2006 to 2016 were collected, and HER2 FISH results were interpreted using the 2013 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: Twenty-nine monosomy cases were identified. The majority of cases were grade 1-2 (65%) and/or ER positive (75%). Concurrent HER2 IHC was performed on 27 cases, with the majority of cases being either negative (59%) or equivocal (37%). The majority of patients had Stage I disease at presentation (57%), followed by Stage II (29%), Stage III (11%) and Stage IV (4%). Twelve patients (41%) had lymph node involvement at presentation, and one patient had distant metastatic disease. Clinical follow-up was available on 26 (90%) cases, ranging from 3-120 months (median = 42). Chemotherapy was administered to 86% of patients. Neoadjuvant chemotherapy was administered in 12 cases (41.4%), five of which also received HER2 targeted therapy (41.7%). All of the cases receiving neoadjuvant therapy without HER2 targeted treatment had a decrease in tumor size with therapy, ranging from a 6% decrease to one with a complete pathologic response. Of the cases that had HER2 targeted neoadjuvant chemotherapy, 2 had a partial response with decrease in tumor size ranging from 29-98%, 2 had an increase in tumor size during treatment, and one patient with Stage IV disease at diagnosis died with no clinical response to therapy. Overall the patients with ER negative and low ER expression had better response to therapy. All but one patient were alive at follow up, and 2 patients had recurrent disease.
Conclusions: Although addition of HER2 targeted neoadjuvant chemotherapy does not appear to add additional benefit to treatment response, this study is limited by the small number of cases. We suggest consideration about whether to treat with chemotherapy plus HER2-targeted therapy be based on correlation with all clinicopathologic characteristics, on a case-by-case basis.

112 Breast Cancers with HER2 and CEP17 "Co-Amplification" by FISH: Pathologic Features and Clinical Outcomes

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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). Evidence suggests that this results most frequently from "co-amplification" of both regions of chromosome 17. Current HER2 testing guidelines consider these patients eligible for HER2 targeted treatments. However, little is known about their clinicopathologic features and clinical outcomes.
Design: Breast cancers with HER2 FISH results from 2006 to 2016 were collected, with HER2 FISH interpreted using the updated 2013 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: Within the 10 year interval, 41 co-amplified cases were identified. The majority of cases were grade 3 (71%) and/or ER positive (64%). Concurrent HER2 IHC was performed on 38 cases, with 71% of cases being IHC positive, 21% IHC equivocal and 8% IHC negative. Clinical stage at presentation varied with 44% stage I, 23% stage II, 23% stage III, and 10% stage IV. Clinical follow-up was available on 35 (85%) of cases, ranging from 3-120 months (median = 44). The rate of metastatic and recurrent disease was high at 59% and 17% respectively, with death from recurrent disease occurring in 2 patients. Neoadjuvant therapy was administered in 13 cases, of which 69% received HER2 targeted therapy in the neoadjuvant setting (9 cases). All of the patients who received HER2 targeted neoadjuvant therapy had a partial (56%, 5 cases) or complete pathologic response (44%, 4 cases). In contrast, the group whose neoadjuvant regimen did not include HER2 targeted treatment experienced less of a response to therapy (three patients had a partial response and one had no response).
Conclusions: Although the sample size is small, overall our results support the current classification of co-amplified cases as HER2 amplified with consideration for HER2-targeted treatment.

113 Breast Cancer Subtype Subsequent to a Benign Breast Biopsy Among African Americans

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Background: Most clinical models to estimate risk of invasive breast cancer (BC) include history of benign breast disease (BBD) as a covariate, as these women represent a higher risk group compared to the general population. A better understanding of the association between BBD and BC is necessary to improve the utility of these risk models, particularly with respect to tumor subtype. This may be especially important for African American (AA) women who are more likely to present with aggressive cancers compared to white women. Here we present tumor subtypes from a higher risk cohort of AA women with a history of BBD.
Design: Benign breast biopsies from 3,737 AA women with BBD diagnosed from 1997-2010 were examined for 14 benign features, and followed for subsequent BC using medical records and data from the SEER database. IHC analysis was performed for the following 6 markers: ER, PR, HER2, Ki-67, epidermal growth factor receptor (EGFR) and cytokeratin 5/6 (CK 5/6) in order to categorize the subsequent BC by subtype. Briefly, ER and PR were utilized to classify tumors as luminal or non-luminal, with further classification made based HER2. Luminal tumors were also classified by Ki-67 expression, and triple negative tumors (ER/PR/HER2 negative) were further classified based on expression of either CK5/6 or EGFR, resulting in 6 categories.
Results: 203 women (5.4% of the total cohort) with a subsequent BC were identified over a mean follow-up time of 12.3 years (range: 4.0 – 17.9). Analysis of all 6 markers is complete for 104 tumors (51.2%). The majority of the subsequent cancers were invasive (n=72, 69.2%). Most of the invasive tumors were luminal B, HER2-, high Ki-67 ≥14% (37.5%), followed by luminal A (31.9%), triple negative (19.4%), non-luminal, HER2+ (6.9%) and luminal B, HER2+, any Ki-67 (4.2%). Of the 14 triple negative cancers (19.4%), 8 were negative for CK5/6 and EGFR (5 negative phenotype, 57.1%) and 6 were core basal (42.9%). Among the 32 *in situ* tumors, the majority were luminal A (n=26, 81.3%), followed by luminal B, HER2- (n=5, 15.6%) and there was a single tumor classified as 5 negative. Compared to population-based SEER data from 5,268 AA women with invasive BC and available data on 3 markers (ER, PR, and HER2) diagnosed in 2010, our cohort is similar with respect to tumor subtype.
Conclusions: The women with a previous benign breast biopsy in our cohort who develop a subsequent BC have subtypes that are similar to the general AA population in the US. Thus, our BBD cohort represents the full spectrum of invasive BC with respect to subtype, including triple negative tumors.

114 Characterization of a Novel Immunohistochemical Antibody Against HER-2 with Neutralizing Properties

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Background: HER-2 (human epidermal growth factor receptor 2), a transmembrane tyrosine kinase proto-oncogene, has been shown to be amplified in 10 to 20% of breast cancers. While the overexpression of HER2 is an independent adverse prognostic factor, targeted therapies such as trastuzumab have significantly improved patient outcome. However, a subset of tumors either do not respond to current therapies or become resistant. Identification of novel detection markers and/or treatment options is critical for patient management.
Design: Mice were immunized with a GST-fusion of the extracellular domain of HER-2. High throughput immunofluoresecent microscopy was used to rapidly identify robotically-picked hybridoma clones that when added to HER-2 (+) cells in culture for 6 hrs, resulted in decreased AKT phosphorylation (pAKT), a downstream signaling target. Clones that suppressed pAKT were expanded and retested for their ability to inhibit growth of HER-2 (+) cell lines, including a trastuzamab resistant cell line. Western blot (WB) analysis was performed on multiple clones for confirmation. A TMA containing 91 evaluable breast DCIS samples was immunohistochemically (IHC) stained with a clinically-validated HER-2 antibody (Neomarkers, clone AB8) or 103/81A, and staining patterns were compared using the CAP/ASCO scoring algorithm (0, 1+, 2+, 3+). A similar staining pattern is defined as an equal score or no more than a 1+ deviation.
Results: Antibody 103/81A inhibits growth of HER-2 (+) cell lines, and WB analysis confirms specificity. 82 of 91 (90%) breast DCIS samples show similar staining patterns. 7 of 9 discordant cases showed 0-1+ for AB8 and a 3+ for 103/81A.
Conclusions: Using a novel, high throughput immunofluorescent microscopy-based hybridoma screening approach, we present 103/81A, a novel monoclonal antibody against HER-2 that shows a similar staining pattern to a clinically validated HER-2 antibody. More importantly, treatment of live cells with 103/81A decreased HER-2 function and cell number HER-2 (+) cell lines, including a trastuzamab-resistant line. The use of 103/81A may provide an additional diagnostic and therapeutic option in patients with HER-2 (+) breast carcinomas. Furthermore, this pilot approach to identifying functional antibodies during primary/secondary hybridoma screening serves as a proof of principle approach to rapidly generate potentially therapeutic antibody reagents for any cell surface oncotargets.

115 Long-Term Clinical Outcome in 41 Cases of Low-Grade Adenosquamous Carcinoma of the Breast

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Background: Low-grade adenosquamous carcinoma (LGASC) is a variant of metaplastic carcinoma known to be associated with a favorable prognosis. Although locally aggressive, LGASC has not been found to readily metastasize or lead to significant mortality. However, these observations are sparsely documented in the literature due to rarity of this entity and consequently, the inability to study a sizable cohort with long term follow up. We aim to further elucidate the long-term behavior of this rare entity using the largest known cohort of LGASC cases.

Design: 46 cases of LGASC were identified in our files between 2000 and 2011. Cases with slides available (n=28) were reviewed for diagnostic accuracy and morphologic features including degree of squamous differentiation, associated calcifications, stromal characteristics and presence of associated lesions were noted. Clinical follow-up including additional surgical or diagnostic procedures, treatment, and death due to disease was obtained.

Results: The majority of patients (41/46; 89%) presented with a mass. In 41% (19/46) of cases there was an associated benign lesion by report and/or review, most commonly a sclerosing papillary lesion (n=11). The majority of cases showed a cellular stroma (n=16), however 8 were notable for alternating patterns of hyalinized, acellular stroma and cellular stroma. 64% (18/28) of cases showed minimal squamous differentiation, while moderate (29%) and marked (7%) differentiation were seen in the remaining cases. Lymphoid aggregates were present in 71% (20/28) of cases. Follow up was available for 41 cases. The average length of follow up was 98 months. 4 patients developed local recurrences, which occurred on average after 54 months of initial excision (7-100 months). Lymph nodes were sampled in 23 patients, all of which were negative for metastatic disease. 3 patients received adjuvant radiotherapy and 2 patients received chemotherapy. None of the patients developed metastatic disease. There were 4 deaths within the follow-up period, however, none were attributable to LGASC.

Conclusions: Our findings confirm the long held belief that patients with LGASC experience an excellent clinical outcome and very unlikely to develop lymph node or distant metastases. To our knowledge, this is the largest cohort of LGASC with long-term clinical outcome in recent years. Additionally, an alternating pattern of hyalinized, acellular and cellular stroma in LGASC has not been previously described in the literature and may have important diagnostic implications on limited samplings such as core biopsies.

116 H3K27me3 Expression and X Chromosome Inactivation in Breast Carcinoma

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Background: Histone 3 trimethylation at lysine 27 (H3K27me3) is an epigenetic modification associated with gene silencing, loss of which has been described in tumorigenesis. H3K27me3 is one mechanism by which one X chromosome is normally inactivated in female cells, creating the Barr body (BB). H3K27me3 expression in breast carcinoma (BC) has not been fully studied; the potential associations with histologic features, biomarkers and outcomes are unknown.

Design: Immunohistochemistry with H3K27me3 rabbit monoclonal antibody was performed on tissue microarrays with 181 total BC, including ER+, HER2+ and triple negative (TN) cases. Expression was scored using H score and categorized into 3 groups: <70, 70-180 and >180; BB were counted. Clinical, histologic and outcome data were analyzed using univariate and multivariate statistics.

Results: On univariate analysis, BB loss was associated with TN subtype and increasing Scarff-Bloom-Richardson (SBR) grade ($p=0.001$, $p<0.0001$); this association was seen with nuclear pleomorphism ($p=0.02$), mitotic index ($p<0.001$) and tubule formation ($p=0.06$). The correlation with TN subtype persisted in multivariate analysis ($p=0.01$). Low H3K27me3 expression was also associated with increasing SBR grade and TN subtype in multivariate analysis ($p=0.02$, $p=0.005$). No association was seen between BB status or H3K27me3 expression and lymphovascular invasion or lymph node status. Among TNBC, a Cox proportional hazards model showed that LVI and LN status, but not BB status or H3K27me3 expression, were associated with BC-specific survival.

Conclusions: BB loss and low H3K27me3 expression are associated with TN subtype and SBR grade. However, among TNBC, neither BB nor H3K27me3 expression were independently correlated with BC-specific survival. These findings raise the possibility that the effects of H3K27me3 are likely involved early in tumorigenesis rather than metastasis.

117 The Molecular Mechanisms of Parity Driving Breast Cancer Etiology

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Background: Reproductive factors are among the most well established risk factors for breast cancer. Parity has been shown to decrease the risk of estrogen (ER) and progesterone (PR) receptor-positive breast tumors and to be inversely associated with breast cancer predictors such as mammographic density.

However, the molecular mechanisms associated with the reduced risk of ER positive breast cancer in parous women are poorly understood. In this study, we aimed at understanding the gene expression differences induced by parity.

Design: We examined the differential gene expression of breast carcinomas and of normal adjacent breast tissue in 596 women part of the Nurses' Health Studies. Primary analysis was performed according to the parity status (as a continuous variable). All analyses were stratified by ER status and adjusted for age of diagnosis, year of diagnosis, post-menopausal hormone therapy use, alcohol intake and microarray batch. Secondary analysis also included the influence of breastfeeding in gene expression in parous women as well a Weighted Gene Correlation Network Analysis (WGCNA) for breast interactome analysis. Additional data from TCGA BRCA cohort on OBGYN history, including parity is available for planned validation of the findings.

Results: Several genes were differentially expressed according to parity in ER positive carcinomas (n=11 genes, FDR<0.05; n=306, FDR<0.25). The top differentially expressed genes included prostaglandin E receptor 1 (PTGER1) and exosome component 6 (EXOSC6), a tumor specific epitope in breast cancer. No significant differentially expressed genes were identified in ER negative cases, in either the carcinoma or normal-adjacent regions. Gene set enrichment analyses (GSEA) suggested that ER positive carcinomas have altered response to estrogen and the existence of an increased inflammatory response in the normal adjacent breast tissue, in women with greater number of children.

Conclusions: These molecular insights further elucidate the effect of parity in breast cancer etiology in post-menopausal women. Our findings suggest that parity could modulate the inflammatory response in ER positive breast carcinomas.

118 Loss of *XIST* – The Potential Breast Cancer X Factor

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Background: It is known for over half a century that breast cancers may lack a Barr body, the heterochromatic inactive X chromosome (Xi) present in normal female cells, but it is unclear if this loss is due to genomic or epigenetic instability. X-inactive specific transcript (*XIST*), a mammalian long non-coding RNA, is required for X chromosome inactivation early in embryogenesis. As XIST is also expressed in adult females, it has been proposed that loss of *XIST* could lead to the Xi reactivation and potentially double dosage of X genes that has been linked to mammary gland tumorigenesis. In this study we assess *XIST* status in a large cohort of patients as a marker of epigenetic dysregulation of the X chromosome and potential indicator of an aggressive phenotype in breast cancer.

Design: Colorimetric RNA *in situ* hybridization (View RNA, Affymetrix, CA) was used to visualize *XIST* in formalin-fixed paraffin-embedded tissue microarrays from 224 annotated breast cancer samples from female patients. *XIST* expression was semiquantitatively scored as *XIST*-low or *XIST*-high by assessing the density and intensity of Fast Red signal dots. *GAPDH* expression was used as housekeeping gene control. The Cancer Genome Atlas (TCGA) via cBioPortal was used to validate the findings. SSPS software was used for statistical analysis.

Results: *XIST* was identified in stromal cells in all cases, and served as internal control. Of the 186 evaluable cases *XIST* expression in the neoplastic cells was low in 51 samples (27%). Low *XIST* correlated with higher histological grade ($p=0.028$); 71% tumors lacking *XIST* expression were grade 3 carcinomas. In addition, there was a higher proportion of ER/PR/HER2 negative tumors among *XIST*-low samples (43%) compared to *XIST*-high cancers (28%), ($p=0.036$). The TCGA data supported the findings: ER positive breast cancers had significantly higher *XIST* expression than ER negative and triple negative tumors (mean 8707 vs 5475 and 5271, z score >2, $p<0.0001$).

Conclusions: Breast cancers with low *XIST* are more frequently poorly differentiated and triple negative tumors. Although defects in XIST likely represent only part of the complex epigenetic changes that affect the X chromosome in breast cancer, they might reflect a broader compromise of the heterochromatic compartment in aggressive breast tumors.

119 ADH, the Precursor to Low Grade DCIS: Is It Time to Consider Active Surveillance for Patients Diagnosed with ADH on Core Needle Biopsy?

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Background: Atypical ductal hyperplasia (ADH), a precursor to low grade ductal carcinoma in situ (LG DCIS), is composed of the same monotonous epithelial cells but does not meet quantitative criteria for LG DCIS. The standard treatment of ADH diagnosed on core needle biopsy (CNB) is surgical excision for the purposes of preventing progression to DCIS and to rule out the presence of DCIS in cases of inadequate CNB sampling. Patients with DCIS have excellent survival rates. Given that recent trials are evaluating whether LG DCIS can be managed by active surveillance rather than surgery, our current standards for surgically excising ADH on CNB may not be optimal treatment. The purpose of this study was to determine the benefit of surgical excision for a CNB diagnosis of ADH.

Design: We retrospectively reviewed pathology and radiology reports of individuals diagnosed with ADH on CNB who underwent a subsequent excision over a 10 year period between 2006 and 2016. Pathology and radiology data were recorded to include mammographic findings, diagnosis on CNB, diagnosis on excision, nuclear grade and hormone receptor (HR) status of DCIS, and combined histologic grade, HR status, HER2 status and tumor stage of invasive carcinoma.

Results: A total of 80 cases were diagnosed as ADH on CNB. On excision, 33 (41%) remained ADH, 24 (30%) had no residual atypia, 18 (22.5%) were DCIS, and 2 (2.5%) were invasive carcinoma. The remaining 3 (4%) patients were diagnosed with focal ADH on CNB and opted for active surveillance instead of surgical excision. The overall upgrade rate was 25%. Of upgraded cases to DCIS, 13 (72%) were low nuclear grade, 5 (28%) were intermediate nuclear grade, zero were high nuclear grade, and all cases were HR positive. Of the 2 upgraded cases to invasive carcinoma, both were diagnosed on CNB as ADH bordering on LG DCIS. The mammographic lesion was a mass in one case and calcifications in the other. Both were diagnosed as HR positive/HER2 negative invasive ductal carcinoma, one was histologic grade 1 with a stage of pT1a, and the other was grade 2 and pT1c.

Conclusions: The majority of cases showed either no residual atypia or remained ADH on excision. The majority of cases upgraded to DCIS were low nuclear grade. Overall, our results suggest that active surveillance rather than surgical excision is a reasonable option for patients diagnosed with ADH on CNB. Mammographic findings and severity of ADH on CNB should be taken into account when deciding on optimal treatment for each individual patient.

120 Mitotic Activity in Benign and Malignant Fibroepithelial Lesions

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Background: Classification of fibroepithelial lesions is based on features including stromal cellularity, stromal mitotic activity, stromal overgrowth and cytological atypia. Brisk mitotic activity is associated with a diagnosis of phyllodes tumour. The proportion of benign, otherwise typical, fibroadenomata bearing low level mitotic activity is not well documented.

Design: The laboratory information system database was searched for all cases coded as 'Fibroadenoma' and 'Phyllodes tumour' from 2008 to 2013. Retrieved case reports were analysed for diagnosis, mitotic activity, tumour border, stromal cellularity, stromal overgrowth and the presence of malignant heterologous features. The data was compiled and analysed with Microsoft Excel. Clinical information and follow up details on relevant cases were obtained.

Results: A total of 1233 fibroepithelial lesions were identified from 259 excisions and 974 biopsies. 64 of these were found to be mitotically active (5.19%). 32 were classified as benign fibroadenomata, 14 were benign phyllodes tumours, 10 were borderline phyllodes tumours and 8 were malignant phyllodes tumours.

81.3% of phyllodes tumours had mitotic counts ≥2/10HPF (N=26/32) and 0.4% of fibroadenomata had mitotic counts ≥2/10HPF (N=5/1201). All patients were followed up with clinical examination and radiology. Five patients (8%) were subsequently found to have recurrent disease. These comprised two fibroadenomata, one benign phyllodes tumour, one borderline phyllodes tumour and one malignant phyllodes tumour. All five recurrences were histologically similar to and occurred on the same side as the original lesions.

Conclusions: Occasional mitotic figures may be identified in benign fibroepithelial tumours, including fibroadenomata. Mitotic activity should be considered with other parameters, most importantly stromal hypercellularity, cytologic atypia, stromal overgrowth, presence/absence of necrosis and tumour border to minimise the risk of misclassification. Recurrence is a well documented complication and is of clinical concern even in benign fibroadenoma and benign or borderline phyllodes lesions which represented 80% of recurrences in this cohort of patients.

121 Evaluation of the Breast Myoepithelial Cell Layer by Physical Expansion of Tissue Microarrays

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Background: Expansion microscopy (ExM) is a novel strategy for studying cell and tissue samples by physically rather than optically magnifying them. In ExM, tissues are isotropically expanded by embedding them in a dense swellable polymer which binds key biomolecules or fluorescent labels to the polymer network. Samples are then mechanically homogenized and swelled, so that they can be imaged with nanoscale (e.g., ~70 nm) resolution on conventional microscopes (Chen F, Science 2015). This method, which has not been previously applied to human breast tissue samples, could be of great value in permitting a more detailed evaluation of breast lesions than achievable by conventional light microscopy. In particular, the myoepithelial cell layer is thought to have a critical role in regulating the progression of ductal carcinoma in situ (DCIS) to invasive breast cancer. These cells have previously been shown to exhibit molecular and immunophenotypic abnormalities in DCIS, but a high resolution evaluation of the myoepithelial cell layer in DCIS could provide new insights into alterations associated with tumor progression.

Design: We optimized the ExM chemistry, labeling, and imaging methodologies to enable ExM to be used for morphological and protein imaging and analysis of tissue microarrays (TMAs). We expanded one FFPE TMA H&E stained slide containing 102 cores of normal breast tissue, DCIS and invasive breast cancer by removing the coverslip, rehydrating the tissue, staining the slide with several fluorescently-labled antibodies and expanding it. Nuclei were stained with DAPI; stromal and myoepithelial cells were stained with antibodies to vimentin, and actin, respectively.

Results: By using this adapted ExM protocol, we successfully expanded one human FFPE TMA containing a spectrum of breast lesions, including normal breast, DCIS and invasive breast cancer ~4.5x in linear dimension. The expanded tissue enabled a more detailed interrogation of the myoepithelial cell layer from both normal breast tissue and DCIS samples than possible by conventional histologic and immunohistochemical evaluation. Our findings indicate that ExM can be applied to the large amount of archival pathology samples to enable super-resolution optical investigation of morphology and protein expression/localization in TMAs with conventional fluorescent microscopy.

Conclusions: In this proof of concept study, we have shown that ExM can be applied to TMAs containing H&E stained breast tissue samples for high resolution evaluation of morphologic and immunophenotypic features.

122 Analytical and Clinical Performance of Monoclonal Antibodies 1E2, 1A6, and PgR636 in the Detection of Progesterone Receptor (PR) in Breast Cancer

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Background: Hormone receptor (HR) immunohistochemistry (IHC) results predict benefit from endocrine therapy and provide prognostic information in luminal-type breast cancers. The goal of this study was to compare the analytical and clinical performance of three different progesterone receptor (PR) monoclonal antibodies.

Design: The binding kinetics for PR clones 1E2, 1A6, and 636 were evaluated on a Biacore T200 using synthetic peptides derived from previously identified epitopes [Virchows Arch (2016) 469 (Suppl 1):S1–S346]. With IRB approval, 313 cases of HR-positive, HER2 negative invasive breast carcinoma were identified over a 9 year period: 90% Stage I-II, 76% received endocrine therapy, and 37% received chemotherapy. The original hormone receptor results were: 182 (58.1%) ER+/PR+, 57 (18.2%) ER-/PR-, 67 (21.4%) ER+/PR-, and 7 (2.2%) ER-/PR+. The overall recurrence rate was 7.92% (25/313) with 19 distant recurrences, 5 local, and 1 of unknown type. Formalin-fixed paraffin-embedded (FFPE) tumors were stained with 1E2, 1A6, and PgR636 and scored by two pathologists using ASCO/CAP guidelines.

Results: Binding kinetic analysis indicates a 5X greater affinity for the PR 1E2 clone compared to PgR 636, due primarily to the observed affinity dissociation rate. When categorized as positive or negative using current ASCO/CAP criteria, the results with all three clones were concordant in 292/313 (83.2%). Of 313 breast cancer cases, 5 (1.6%) were PR-positive with 1E2 and negative with either PgR 636 or 1A6. All 5 tumors were stage II-III, ER-negative, high-grade invasive ductal carcinomas. None of these patients received endocrine therapy, 3/5 received chemotherapy, 2/5 received radiation therapy and 2/5 had a distant recurrence within 36 months.

Conclusions: Among three PR monoclonal antibodies, 1E2 showed the highest affinity for PR in binding kinetic analysis. In a minority of cases (1.6%), 1E2 converted ER-/PR- patients to ER-/PR+, potentially making them eligible for endocrine therapy. Expanding the cohort size and extending the follow-up period will be required to determine the clinical significance of these findings.

123 Next-Generation Sequencing Profiling of Circulating Tumor Cells in Metastatic Breast Cancer Patients Reveals Mutational Heterogeneity

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Background: Metastatic breast cancer (MBC) after endocrine therapy harbors DNA alterations thought to arise from selective pressure on cancer cells exerted by the treatment. Somatic mutations in cancer have been identified in circulating cell free plasma tumor DNA (ptDNA), however, the mutational profile of DNA extracted from circulating tumor cells (CTC) is less well characterized.

Design: We studied 13 patients with MBC who had ≥5CTC/7.5 ml whole blood (WB), and had at least one CTC with high quality DNA (Ampli1™ QC kit). CTCs were enriched from WB (CellSearch©) and purified from white blood cells (WBC) (DEPArray™). DNA from individual and pooled CTCs and WBCs was isolated and subjected to whole genomic amplification (Ampli 1™ WGA) and genotyped by multiplexed PCR-based next generation sequencing with the Oncomine Comprehensive Panel (OCP) on the Ion Torrent Proton. Slected mutations were validated by Sanger sequencing. Exome sequencing of research biopsies of metastatic tissue was previously performed on the Illumina HiSeq 2500 platform under the MI-ONCOSEQ effort.

Results: Mutations were detected in patient individual CTCs with concordant results between CTCs and CTC pools. Several mutations were detected in CTCs that were also found in the research biopsy as well as in cell free tumor DNA, among them mutations in the estrogen receptor (ESR1, Y537S). In addition, novel alterations were found in CTCs compared to research biopsies in nine of 13 patients. Among these was one CTC (out of 29 sequenced for that patient) that harbored a previously uncharacterized ESR1 mutation. In two patients, two potentially actionable mutations (*PTCH1* and *NOTCH1*) were found in CTC-DNA but not in tissue-DNA. Two patients had lobular carcinoma and as expected harbored somatic, deleterious CDH1 (E-cadherin) mutations (frameshift and nonsense) in both metastatic biopsy and CTC-DNA. Individual patients showed mutational heterogeneity among their CTCs.

Conclusions: We performed next generation sequencing on CTCs from MBC patients and confirmed several mutations from the research biopsy and cell free DNA data. In addition, we detected mutations not present in the research biopsy and observed heterogeneity among CTCs in individual patients.

124 Cell Cycle Aberrations and Diagnostic Utility of Rb and Cyclin D1 Immunohistochemistry in Mammary Myofibroblastomas

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Background: Mammary myofibroblastoma (M-MFB) is a benign neoplasm of breast stromal myofibroblasts. The diagnosis can be challenging, particularly on core needle biopsy, where the differential diagnosis includes fibroepithelial lesions, pseudoangiomatous stromal hyperplasia, lobular carcinoma, and metaplastic carcinoma.

Cytogenetic studies have revealed monallelic loss of chromosome 13q14, which contains the *RB1* gene that codes for retinoblastoma protein (Rb). MFBs originating outside the breast display loss of nuclear labeling for Rb by immunohistochemistry (IHC). Whether IHC for Rb is a useful diagnostic tool for M-MFBs remains to be evaluated. To clarify the role of cell-cycle mediators and evaluate their diagnostic utility, we systematically studied the expression of Rb, cyclin D1, p16 and PTEN in M-MFBs.

Design: Thirty-two cases of histologically confirmed M-MFB were identified from the pathology archives, and tissue from 18 cases was available for construction of a tissue microarray (TMA) consisting of five 1.4 mm cores per tumor. Whole slide sections of the remaining 15 cases were evaluated. Clinicopathologic data including IHC performed at the time of diagnosis were recorded, and cases were labeled by IHC for Rb (clone G3-245, mouse monoclonal, BD Pharmingen), cyclin D1, p16, and PTEN. Rb, cyclin D1 and PTEN was scored as percentage nuclear labeling, with loss defined as was <10% of tumor cell nuclei labeling with intact internal controls. IHC for p16 was scored as percentage cytoplasmic and nuclear labeling.

Results: The majority of patients (69%) were female, and the average age at diagnosis was 62 years (range, 30-89). Follow-up data was available on 12 (38%) patients, with no reported recurrences. In total, 31% (n=10) of tumors displayed Rb loss, while 58% (n=18) displayed cyclin D1 loss. The majority of cases exhibited focal labeling for p16 (80%). Six tumors demonstrated high p16 expression (between 50-95% tumor nuclei labeling), but only one was p16-high and Rb-. Nuclear PTEN labeling was intact in 97% of tumors.

Conclusions: Although M-MFBs have a common cytogenetic finding of monallelic deletion of RB1, the majority shows intact, although heterogenous, nuclear labeling for Rb by IHC. Loss of nuclear cyclin D1 labeling is more sensitive for a diagnosis of M-MFB and may be a useful diagnostic adjunct. Additional studies are warranted to correlate the gene allelic status with the IHC labeling patterns.

125 EZH2 Overexpression in Breast Cancer: Strong Correlation with HER2 Overexpression

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Background: EZH2 (Enhancer of zeste homolog 2) is an enzymatic subunit of polycomb repressive complex. Recent studies have shown that EZH2 overexpression may be associated with poor clinical outcomes in breast cancer. In addition to being a prognostic factor, EZH2 inhibitors have shown early signs of promise in recent clinical trials, suggesting that EZH2 is a potential therapy target. However, the relationship between EZH2 overexpression and the clinicopathologic features of breast cancer has still not been thoroughly evaluated.

Design: We investigated the relationship between EZH2 expression and the clinicopathologic features of breast cancer (histologic grade, tumor stage, and tumor biomarker expression) on tissue microarray sections of 278 cases of invasive breast carcinoma. EZH2 expression was assessed by immunohistochemical analysis using monoclonal EZH2 antibody; only nuclear staining was considered positive expression. The staining intensity (0, 1, 2, or 3) and percentage of positive tumor cells (0%-100%) were estimated and the H score of EZH2 expression was calculated (H score = staining intensity x percentage of positive tumor). EZH2 was considered to be overexpressed when the H score was ≥60.

Results: Interpretable EZH2 staining was observed in 248 of 278 cases (89.2%). Eighty-two of these 248 cases showed EZH2 overexpression (33.1%; H score range, 60-285, mean, 170.4]). High EZH2 expression was significantly associated with HER2 overexpression (p<0.0001) and high histologic grade (p<0.0001); in contrast, ER positivity was inversely related to high EZH2 expression (p<0.0001, Table 1). High EZH2 expression was not significantly associated with pathologic stage or with the presence of regional lymph node metastasis (p=0.08 and 0.85, respectively).

Table 1. Relationship between EZH2 overexpression and clinicopathologic features

Pathologic features	N (%)	Mean H score of EZH2	p value
Histologic grade			<0.0001
3	81 (32.7)	131.9	
2	123 (49.6)	39.5	
1	44 (17.7)	24.3	
HER2 status			<0.0001
+	23 (9.3)	155.2	
-	225 (90.7)	58.0	
ER/PR status			<0.0001
+	215 (86.7)	59.7	
-	33 (13.3)	153.8	

Conclusions: EZH2 overexpression is strongly correlated with more aggressive pathologic features of breast cancer, including high histologic grade and HER2 overexpression. These findings suggest that EZH2 overexpression is an important prognostic factor in breast cancer. Future investigations of the potential utility of EZH2-targeted therapy for breast cancer are warranted.

126 "Pure Intralymphatic Carcinoma" Following Neoadjuvant Chemotherapy for Breast Carcinoma Is Associated with Poor Prognosis and Should Not Be Considered Pathologic Complete Response

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Background: Pathologic complete response (pCR) following neoadjuvant chemotherapy (NAC) correlates with long-term survival and is a primary endpoint for clinical trials. Rarely, post-NAC specimens reveal residual carcinoma confined to lymphatics without stromal invasive carcinoma, i.e., "pure intralymphatic carcinoma" (PIC) (*Am J Surg Pathol* 2009;33:256-63), and has been associated with an aggressive course. PIC is not recognized in AJCC staging guidelines and has not been addressed in neoadjuvant trials. We set out to further characterize this pattern of residual disease.

Design: Patients who underwent surgery following NAC for invasive carcinoma were identified (2005-2016). Slides were reviewed. D2-40 and CD31 immunostains were performed to confirm PIC in equivocal cases. Clinical data were reviewed.

Results: Of 293 post-NAC cases, 6 (2%) had PIC, identified as one or multiple clusters of intralymphatic carcinoma within the tumor bed without associated invasive carcinoma. All 6 corresponding pre-NAC biopsies showed poorly-differentiated carcinoma [triple-negative: n=3; HER2+: n=3 (2 ER+, 1 ER-)]. Five patients had pretreatment biopsy-proven axillary lymph node involvement and one had distant metastasis at presentation. Following NAC (AC-T ± trastuzumab), a marked clinical response was seen in all patients. 3 patients underwent mastectomy and 3 had lumpectomy. Two patients (33%) had residual carcinoma in lymph nodes. All 6 patients received post-operative radiotherapy. One patient died of pulmonary embolism 2 months following surgery. Of the remaining patients, 3 had distant metastases, and one patient died of disease [mean follow-up 39 months (range: 10-71)].

Age	Pre-NAC tumor	Surgery	Post-NAC LN status	Follow-up
62	1.4 cm; LN+ TNBC	L	yN0	Mets: pleura, brain (5, 7 mo); DOD
46	5 cm; LN+ TNBC	L	yN1a	Mets: skin, pleura (29, 36 mo)
33	5 cm ER+, PR-, HER2+	M	yN0	Met: liver (24 mo)
33	2.5 cm; LN+ ER+, PR+, HER2+	M	yN0	NED (56 mo)
64	2 cm; LN+ ER-, PR-, HER2+	M	yN0	Died of PE (2 mo)
52	3.3 cm; LN+ TNBC	L	yN1a	Met: bone (presentation)

LN, axillary lymph node; DOD, died of disease; L, lumpectomy; M, mastectomy; met, metastasis; mo, months; NED, no evidence of disease; PE, pulmonary embolism; TNBC, triple-negative

Conclusions: PIC following NAC is an uncommon finding. Based on our follow-up data and those published previously, PIC should not be considered pCR (including when lymph nodes are negative following NAC).

127 Correlation of Tumor-Infiltrating Lymphocytes (TILs) with Residual Cancer Burden in Patients Treated with Neoadjuvant Chemotherapy (NAC) for Invasive Breast Carcinoma with a Focus on TILs Assessment in Heterogeneous Cases

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Background: Accumulating evidence has shown TILs to be prognostic in invasive breast carcinoma (IBC) and predictive of response to NAC. The International TILs Working Group has recommended H&E quantification of mean stromal TILs across the tumor for TILs assessment. In a proportion of tumors, the distribution of TILs is heterogeneous with more abundant TILs at the invasive edge of the tumor. The significance of TILs "hot spots" with respect to response to NAC has not been formally studied. We aimed to correlate pre-NAC stromal TILs with residual disease (RD) following NAC with a focus on comparing methods for quantifying TILs in pre-NAC core biopsies.

Design: We identified 170 patients that had surgery s/p NAC for IBC (2005-2016) with H&E slides available from core biopsy and excisions. In core biopsies, mean stromal TILs were calculated from three 20x fields per case when TILs were homogeneous in distribution and from six fields if TILs were heterogeneous. In IBCs with abundant TILs at the invasive edge of the tumor, TILs for the 1 field including the hot spot were assessed separately. Slides from excisions were reviewed to assess RD by the Residual Cancer Burden (RCB) system. RCB was grouped as 0/I: complete response (pCR)/ minimal RD, and II/III: moderate/extensive RD.

Results: Patient age ranged from 26-91 yrs (median: 53). pCR was achieved in 30/170 (17.6%) cases and RCB 0/I in 56/170 (32.9%), and was more frequent in HER2+ and triple-negative tumors (64.4%, 34.3%, respectively). Mean stromal TILs assessment showed cases with RCB 0/I to have significantly greater TILs in pre-NAC core biopsies than those with RCB II/III overall (p=.02) and in ER-/HER2+ tumors (p=.04); however, the association between high TILs (>50%) in core biopsies and response was not significant (p=.15). TILs hot spots were identified in 37/170 (21.8%) core biopsies, and were not more frequently seen in any subtype of IBC. Using TILs values from hot spots cases increased the overall number of high TILs cases to 49/170 (28.8%), and high TILs significantly correlated with response (RCB 0/I) (p=.047) using this method. The degree of pre-NAC stromal TILs correlated with RCB index (continuous variable) by either method (mean TILs, p=.009; hot spot TILs, p=.004).

Conclusions: Our study confirms previous observations of the relationship between pre-NAC TILs and response to NAC. Evaluation of TILs hot spots in pre-NAC core biopsy samples may be relevant in cases with heterogeneous TILs and warrants further study.

128 Prognostic Importance of Ki-67 Assessment in Residual Invasive Breast Carcinoma Following Neoadjuvant Chemotherapy: A Study of 106 Patients with Follow-Up

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Background: Patients with residual disease following neoadjuvant chemotherapy (NAC) for invasive breast carcinoma (IBC) have significantly worse outcomes than those achieving pathologic complete response (pCR). Various clinicopathologic parameters have been associated with poor prognosis in patients with residual disease. Post-NAC proliferation in IBC, as measured by Ki-67, has been shown to be independently prognostic in some studies, while others have not found this association.

Design: We identified patients that had surgery at our institution following NAC for invasive breast carcinoma (2005-2016) with residual invasive carcinoma in the breast (at least T1a) and had at least 36 months follow-up (n=106). Ki-67 staining was performed on one section containing invasive carcinoma from core biopsy and post-NAC excision specimens. High Ki-67 was defined as ≥ 20%. Slides from post-NAC specimens were reviewed to assess residual disease (RD) according to by the Residual Cancer Burden (RCB) system. RCB was grouped as I: minimal RD, II: moderate RD, III: extensive RD.

Results: Patients ranged in age from 25 to 81 yrs (median: 52.5). 47 patients had local excision and 59 patients had mastectomy. RCB classes following NAC were: I: n=9 (8%); II: n=56 (53%), III: n=41 (39%). Patients' tumors were hormone receptor (HR)+/HER2-: n=66 (62%), triple-negative (TNBC): n=17 (16%), HR+/HER2+: n=16 (15%), HR-/HER2+: n=5 (5%), HR+/HER2-equivocal: n=2 (2%). Ki-67 values ranged from 0 to 100% (median: 5, mean: 18.6), and were highest in TNBC (mean: 47.9, p<.001). Overall, 34/106 (32%) tumors showed high Ki-67 staining. At a mean follow-up of 59 months, 40 (38%) patients had distant recurrence. Tumors with high Ki-67 following NAC showed more frequent recurrence overall (p=.01) and in HR+/HER2- tumors (p=.04). Further, Ki-67 was significantly associated with distant recurrence, independent of RCB class, lymph node status, and tumor size [Ki-67 high vs. low, p=0.0045, OR: 4.1 (1.6, 10.9)]. For 41 cases with core biopsy Ki-67 available, 31 (76%) showed a decrease in Ki-67 on excision, while 10 (24%) showed an increase or equal Ki-67 value. Neither core biopsy Ki-67 nor change in Ki-67 were associated with recurrence in this smaller subset of cases.

Conclusions: Ki-67 evaluation in post-NAC specimens is an independent prognostic factor and may help guide further management of patients not achieving pCR, particularly those with HR+/HER2- tumors.

129 Validation of the Singapore Nomogram for Outcome Prediction in Breast Phyllodes Tumors in a Large Patient Cohort

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Background: The grading of breast phyllodes tumors, based on semi-quantitative parameters, is often impeded by tumor heterogeneity and poor inter-observer concordance. The Singapore nomogram for outcome prediction in breast phyllodes tumors uses histological parameters and surgical margin status to estimate recurrence free survival (RFS) of affected individuals, providing pathologists and clinicians with an aid to patient management independent of tumor grade. We aim to validate the nomogram in a cohort of Singaporean patients.

Design: Phyllodes tumors treated in a women's hospital from 2006 to 2015 were collected. Recurrences and repeat procedures (e.g. excision following diagnostic biopsy) were collated and corroborated with clinical follow-up data. Histology review was performed for stromal cellularity, stromal atypia, mitoses per 10 high power fields, type of border (permeative or pushing), presence and type of malignant heterologous elements and surgical margin status. Cases with concurrent malignant or pre-malignant disease were excluded from the validation to minimize confounding influences.

Results: 259 patients were identified. Their ages ranged from 15 to 75 years (median 37.7 years). The median follow-up duration was 1.66 years (range: 0.01 to 10.2 years). 211 tumors were graded benign (81.5%), 30 were borderline (11.6%) and 18 were malignant (6.9%). Local recurrences were present in 13 women (9 benign, 2 borderline and 2 malignant tumors, 11 of which had involved margins in the original excision). The median time to recurrence was 2.3 years. One woman whose tumor recurred subsequently died from her disease; this was the only death due to phyllodes tumor. 19 patients had concurrent malignant or pre-malignant disease (including atypical ductal hyperplasia, atypical lobular hyperplasia, ductal carcinoma in situ, lobular carcinoma in situ and invasive carcinoma), and were excluded from the validation.

Univariable Cox regression of 240 patients showed that those with higher mitotic rates, stromal overgrowth and positive surgical margins had increased risks of developing relapse (HR=1.11, 95% CI=(1.06, 1.17)); HR=3.61, 95% CI=(0.99, 13.16); HR=12.92, 95% CI=(2.86, 58.38) respectively). Patients with higher nomogram scores also had significantly greater risk of developing relapse (HR=1.07, 95% CI=(1.04, 1.11)), with concordance index of 0.863.

Conclusions: The Singapore nomogram was able to predict RFS in a large cohort of women with breast phyllodes tumors, indicating its role as an adjunct to histological grade in predicting biological behavior.

130 The Prognostic Impact of Synchronous Ipsilateral Multiple Breast Cancer

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Background: According to the current American Joint Committee on Cancer(AJCC) staging system, T stage is determined only by the largest tumor size regardless of heavy tumor burden of multiple breast cancer. We aimed to evaluate if tumor multiplicity had the prognostic value and could be used to subclassify the invasive breast cancer.

Design: We included 5797 patients (mean age, 47years; age range, 21-36 years) with invasive breast cancer who underwent conserving breast surgery or total mastectomy at Samsung Medical Center from 1995 to 2012. For inclusion in the study, patients needed to meet the following criteria: no distant metastasis at the time of diagnosis, no neoadjuvant therapy before surgery, more than follow-up period of 36 months. Median follow-up was 64 months.

Results: Patients were divided into two groups according to multiplicity. Disease-free survival(DFS) was evaluated between patients with single mass (n=4778) and multiple masses (n=1019). In univariate Kaplan-Meier survival analysis, patients with multiple masses had significantly poorer DFS (p=0.0116). Multiplicity and clinico-pathological variables (age, tumor stage, lymph node metastasis, histologic grade, status of the estrogen receptor(ER), and human epidermal growth factor receptor 2(HER2)) were analyzed with multivariate Cox proportional hazards models. The multivariate analysis indicated that tumor multiplicity correlated independently with worse DFS (HR 1.23, 95% CI 1.03-1.47, p=0.021). Other independent factors were age, tumor stage, lymph node metastasis and histologic grade.

Conclusions: Tumor multiplicity is independently associated with worse disease-free survival in patients of invasive breast cancer.

131 Expression of Molecules Involved in Cell-Extracellular Matrix(ECM) Adhesion Is Associated with Characteristic Micropapillary Morphology and Extensive Lymph Node Metastasis of Breast: Next-Generation Sequencing Analysis

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Background: Invasive micropapillary carcinoma(IMPC) of breast is rare subtype of breast cancer, which has tendency of frequent lymph node metastasis. The aim of this study is to characterize genetic profile of IMPC and to get insights how IMPC have specific morphology and specific phenotype.

Design: We performed whole exome sequencing and microarray comparative genomic hybridization (aCGH) on 2 IMPC, targeted deep sequencing on 13 IMPC, and whole transcriptome sequencing on 10 IMPC. The mutation frequency was compared with 537 non-IMPC breast cancer samples. The RNA expression was compared with 119 non-IMPC breast cancer samples and gene set enrichment analysis was performed.

Results: KEGG pathway extracellular matrix(ECM)-receptor interaction gene set was enriched in IMPC (q-value=0.016). The expression of ECM-receptor interaction gene set was correlated with number of lymph node metastasis in 49 non-IMPC samples. In whole exome sequencing, mutated genes possibly affecting ECM-receptor interaction were *ANK1* and *CD44*. In targeted deep sequencing, *PALB2* and *NOTCH1* mutations were more frequent in IMPC than non-IMPC.

Conclusions: Alterations of genes related to cell-extracellular matrix(ECM) adhesion may be related to pathophysiology of invasive micropapillary carcinoma(IMPC) and make IMPC more frequently metastasize to lymph nodes.

132 TSC2 Gene and Protein as a Biomarker for Primary and Metastatic Breast Cancer

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Background: TSC2 (tuberin) is a tumor suppressor associated with mammalian target of rapamycin complex 1 (mTORC1). In this study, we evaluated the prognostic significance of TSC2 protein expression and queried whether genomic alterations (GA) in the *TSC2* gene could guide personalized therapies in 2 separate cohorts of primary invasive (IBC) and metastatic (mBC) breast cancer patients.

Design: FFPE sections from 80 IBCs [63 ductal (IDC) and 17 lobular (ILC)] were immunostained by an automated method (Ventana) using rabbit monoclonal tuberin/TSC2 antibody (clone D93F12, Cell Signaling). Cytoplasmic (cTSC2) and/or membranous (mTSC2) immunoreactivity was scored based on intensity and percent positive cells in tumor (T) and adjacent benign (B) epithelium, then assessed asT>B, T=B, T<B or negative (N). Results were correlated with clinicopathologic variables. Comprehensive genomic profiling (CGP) was performed on a separate cohort of 10,336 mBC using a hybrid-capture, adaptor ligation based next generation sequencing assay to a mean coverage depth of >600X. Tumor mutational burden (TMB) was calculated from a minimum of 1.11 Mb of sequenced DNA as previously described and reported as mutations/Mb.

Results: cTSC2 immunoreactivity was noted as: T>B 64%, T=B 27% and T<B 7%; correlating with menopausal age at diagnosis overall (p=0.05) and within the ER- subgroup (p=0.027). mTSC2 was noted as: N 80%, T>B 18% and T=B 2%; with increased mTSC2 correlating with peri-menopausal age at diagnosis (p=0.043) and high grade (p=0.049) in the ER+ subgroup; while showing a trend toward correlation with ER+ status (p=0.087) and advanced stage (p=0.108) overall and with PR- status (p=0.06), peri-menopausal age at diagnosis (p=0.069), and disease recurrence (p=0.098) within ILCs. On multivariate analysis, advanced stage (p<0.0001) and age at Dx (p=0.005) independently predicted recurrence free survival; while disease recurrence (p<0.0001) and high grade (p=0.025) independently predicted overall survival. CGP revealed that 87 mBC (0.8%) harbored *TSC2* GA. The mean TMB of the *TSC2* altered mBC was 10 mut/Mb. 26% of *TSC2* altered mBC had ≥ 10 mut/Mb and 10 % TMB ≥ 20respectively. In comparison, only 5% of non-*TSC2* altered mBC had ≥10 mut/Mb and only 1% had ≥ 20 mut/Mb.

Conclusions: TSC2 expression is associated with multiple clinicopathologic variables and approaches significance as a prognostic factor in IBC. Inactivating GA in *TSC2* in patients with mBC, although extremely uncommon, are associated with high TMB suggesting that both MTOR inhibitors and immunotherapies could conceivably be useful in the treatment of patients with clinically advanced disease.

133 Histologic Subtypes of Breast Cancer Correlates with Recurrence Score When Divided by Risk Categories

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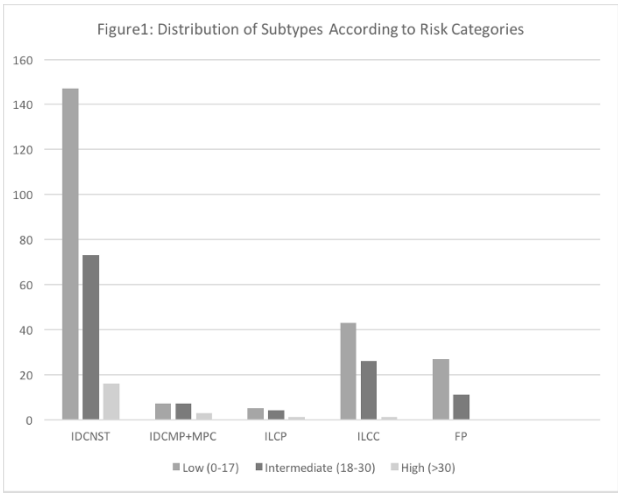
Background: Numerous studies have shown the relation between traditional prognostic markers such as grade, tumor size, hormone receptor status (HRS), Ki-67 proliferation index (PI) and OncotypeDX recurrence score (RS). However, only a few studies have looked at the relation between histologic subtypes (HS) and RS. This study focuses on this relationship.

Design: RS for 371 tumors from 360 patients (355 unilateral and five bilateral) were available. Patients were divided into seven subgroups by HS: invasive ductal carcinoma, no special type (IDCNST); IDC with micropapillary features (IDCMP), pure invasive micropapillary carcinoma (MPC), invasive lobular carcinoma classic type (ILCC); ILC pleomorphic type (ILCP); mixed ductal and lobular carcinoma (DLC) and invasive mammary carcinoma with favorable prognosis (FP) (mucinous, tubular, cribriform, tubulolobular) and three subgroups by risk categories (RC): low (0-17), intermediate (18-30) and high (>30).



IDCNST was compared to special subtypes (SS) according to mean RS and RC. RS was compared to size, grade, lymph node status (LNS), HS and PI.

Results: Of 371 tumors, 236 were IDCNST; 71 ILCC; 21 FP; 17 DLC, 13 IDCMP; 10 ILCP; 4 MPC. There was no statistically significant difference (SSD) between IDCNST and SS in terms of mean RS within grades (p=0.442). There was SSD between IDCNST and SS when divided by RC (p=0.049).

Table1:Comparison of IDCNST to special subtypes of breast cancer according to risk categories.

Histologic Subtype	Oncotype Risk Categories			Total	p
	Low (0-17)	Intermediate (18-30)	High (>30)		
IDCNST	149(63.1%)	71(30.1%)	16(6.8%)	236	
IDCMP+MPC	7(41.2%)	7(41.2%)	3(17.6%)	17	
ILCP	5(50%)	4(40%)	1(10%)	10	0.049
ILCC	43(60.6%)	27(38%)	1(1.4%)	71	
FP	15(71.4%)	6(28.6%)	0(0%)	21	

There was a positive correlation between RS, grade, and PI (p<0.001); and negative correlation with ER and PR expression (p<0.001). There was no SSD between RS and TS, LNS, or age.

Conclusions: The findings of this study suggest a relation between breast cancer histologic subtype and OncotypeDx RS by risk category independent of histologic grade.

134 Validation of the Singapore Nomogram for Outcome Prediction in a US-Based Population of Women with Breast Phyllodes Tumors (PT)

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Background: No grading scheme can reliably predict the behavior of a breast PT. The PT Singapore Nomogram (PTSN) was developed based on data from a cohort of Asian women with PT, and estimates the recurrence free survival (RFS). We examined whether the PTSN is also predictive of RFS in a United States (US)-based patient cohort.

Design: We assessed the histologic features, surgical margin status, and clinical follow-up data of women with PT treated at a US-based tertiary care center between 1990-2014. Kaplan-Meier survival curves were used to estimate local and/or distant RFS, defined as the time from date of surgery to date of first relapse or death from PT or to the last follow-up date for censored cases. Univariate Cox regression (UCR) analysis was performed to evaluate the effects of predictors in the PTSN on RFS. Harrell's c-index was used to assess the probability of concordance between predicted and observed survivals by means of the PTSN (c=0.5 for random predictions; c=1 for a perfectly discriminating model).

Results: Analysis included 76 women with PT. UCR showed that stromal atypia and higher stromal mitotic rate, were each associated with a higher risk of local and/or distant relapse. PTs with stromal overgrowth tended to have a shorter RFS than those without. Malignant PTs had a higher risk of relapse than benign PT. Patients with a higher PTSN score had a significantly higher risk of relapse with a c-index of 0.84.

Table 1. Univariate Analysis of RFS

Factor	No. of patients n=76 (%)	No. of events n=9	HR (95% Cl)	P-value
Diagnosis				0.0139
Benign	45 (59)	2	Reference	
Borderline	15 (20)	2	3.23 (0.45, 23.01)	
Malignant	16 (21)	5	8.10 (1.57, 41.87)	
Overgrowth				<0.0001
Negative	64 (82)	4	Reference	
Positive	12 (18)	5	10.67 (2.79, 40.79)	
Atypia				0.0001
Mild	48 (63)	1	Reference	
Moderate	18 (24)	3	8.75 (0.91, 84.12)	
Marked	10 (13)	5	28.65 (3.32, 247.26)	
Mitoses	76 (100)	9	1.08 (1.04, 1.13)	<0.0001
Margin				0.92
Negative	61 (80)	7	Reference	
Positive	15 (20)	2	1.08 (0.22, 5.21)	
Nomogram	76 (100)	9	1.06 (1.02, 1.11)	0.0007

Conclusions: Analysis of a US-based cohort of women with PT using the Singapore Nomogram yielded a concordance index of 0.84. Our findings support the utility of the PT Singapore Nomogram to estimate the RFS in US women.

135 Is Re-Excision of Benign and Borderline Phyllodes Tumors with Positive Margins Necessary?

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Background: Phyllodes Tumors (PTs) are uncommon breast neoplasms; old series report local recurrence (LR) rates >10%, sometimes with grade progression, and potential for distant metastases. Wide local excision (EXC) of PTs with a surgical margin (SM) ≥1cm is usually recommended to minimize the risk of LR. Nonetheless, management of benign (BPT) and borderline (BLPT) PTs with positive (+)SM varies, and not all patients undergo re-excision. We sought to correlate margin status with LR and grade progression in a recent series of BPTs and BLPTs.

Design: We searched the pathology database for patients with BPT or BLPT, surgically excised at our Center from 1990 to 2014. Clinical records and slides were reviewed.

Results: We identified 59 women with 63 PTs, including 46 (73%) BPTs and 17 (27%) BLPTs. The patient median age was 43 years (range 18-77); a woman had 2 PTs, another 4. The median tumor size was 2.2 cm (range 0.5 - 9.5). Twenty-eight PTs (44%) had (+)SM at first EXC (21 BPTs, 7 BLPTs), but only 12 (8 BPTs, 4 BLPTs) underwent re-excision yielding negative margins. 16 PTs had final (+)SM (13 BPTs, 3 BLPTs). Four of 63 (6.3%) PTs recurred. Two (1 BPT, 1 BLPT) of the 16 PTs (12.5%) with (+)SM recurred. Two of 45 PTs (1 BPT, 1 BLPT) with negative (-)SM also recurred. The LR rate was 4.3% (2/46) in the BPT group and 11. 8% (2/17) in the BLPT group. All LRs had the same grade as the index PT. None of the patients developed distant metastases. Median follow up time for PT with (+)SM that did not recur was 41 months (range 6-217).

	Recurrence n=4 (%)	No recurrence n=59 (%)
Grade of PT n=63		
Benign	2 (4.3)	42 (91.3)
Borderline	2 (11.8)	15 (88.2)
Median age (years)	30	43
Median size (cm)	2.8	2.0
Median follow up time (months)	35	58
Border		
Circumscribed n=45	5 (11.1)	40 (88.9)
Infiltrative n=18	1 (12.5)	17 (87.5)
Stromal Overgrowth		
Absent	4	57
Present	0	2
Mitotic Rate		
<5	2	49
5-9	1	10
>10	0	1
Stromal Atypia		
Mild	1 (25)	51 (86)
Moderate	3 (75)	7 (12)
Marked	0	1 (2)
Margin Status		
Positive	2 (50)	14 (24)
Negative	2 (50)	45 (76)

Conclusions: None of the recurrent PTs in our cohort showed grade progression. The overall LR rate for borderline PTs was 11% and was increased by (+)SM status, which supports re-excision of (+) margins. Our results show a 4% overall rate of LR for benign PTs; the LR rate of benign PTs with (+)SM was <10%. These data suggest that re-excision of benign PTs with (+)SM may not be required.

136 Stromal Density of Tumor-Infiltrating Lymphocytes (TILs): Challenging the 50% Threshold That Defines Lymphocyte Predominant Breast Cancer (LPBC)

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Background: Assessment of TILs as a clinically relevant immunologic marker in breast cancer is becoming more established, particularly in triple-negative breast cancer (TNBC). Typically, thresholds of ≥50 or 60% have been used to define LPBC; however, there is no published evidence to indicate that these cutoffs correlate with long-term clinical outcome in TNBC. We sought to determine a clinically relevant threshold for TILs in a cohort of patients with TNBC with long-term outcome.

Design: Histopathologic assessment was performed on a representative H&E slide of 76 primary invasive TNBC cases. Stromal density of TILs defined as a percentage of intratumoral stroma occupied by mononuclear inflammatory cells over the total intratumoral stromal area was recorded in each case at 5% increments. Statistical analyses using disease-free survival (DFS) and overall survival (OS) (range: 16 to 196 months, mean: 110 months) as primary endpoints were performed to determine clinically relevant thresholds of stromal density to define LPBC from non-LPBC.

Results: Using DFS as the primary endpoint in our cohort of TNBC, the ideal threshold of stromal density of TILs for LPBC based on the lowest P value (0.0006) was ≥32.5%. In addition, a stromal density threshold of ≥50% to define LPBC was significantly associated with DFS (P=0.0149). When using OS as the primary endpoint in our cohort of TNBC, the ideal threshold of stromal density of TILs for LPBC based on the lowest P value (0.0308) was ≥57.5%. However, using a stromal density threshold of ≥50% to define LBPC was not found to be significantly associated with OS (P=0.0599). Additionally, each 5% increase in stromal density of TILs was significantly associated with improved DFS and OS in TNBC [0.04; 95% confidence interval (CI) 0.01–0.33, P=0.0025 and 0.09; 95% CI 0.01–0.69, P=0.0206, respectively].

Conclusions: We found that stromal density of TILs correlates significantly with long-term clinical outcome in a dose-response manner in TNBC. Our findings help validate using a higher threshold of a ≥60% for stromal TIL density (instead of 50%) to morphologically classify LPBC in TNBC for survival prognostication. Additionally, our results suggest that using a threshold of >30% for may be useful in predicting recurrence.

137 Tumor-Infiltrating Lymphocyte (TIL) Assessment Distilled into Two Binary Parameters in Triple-Negative Breast Cancer (TNBC)

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Background: TILs provide prognostic and potentially predictive value, particularly in TNBC. The 2014 International TILs Working Group guidelines for TIL assessment include multiple parameters including quantification of TILs as a continuous variable, TIL location (stromal versus intratumoral), and grading extent of stromal lymphocytic aggregates. Such multi-parameter assessment is onerous for the practicing pathologist and prone to considerable inter-observer variability and low reproducibility. We sought to identify a simplified method to assess TILs at the microscope while maintaining prognostic value in TNBC.

Design: Quantification of mononuclear TILs was performed on a representative H&E slide from 76 cases of primary invasive TNBC. Percent stromal mononuclear TILs (sTILs) within the entire tumor area were estimated. Tumors were defined as LPBC or non-LPBC using a cutoff of ≥50% sTILs. TIL location was recorded as only sTILs or both sTILs and intratumoral TILs (iTILs). Stromal lymphoid aggregates were classified as no aggregates, rare aggregates, well-developed aggregates or aggregates with germinal centers. Statistical analyses were performed using disease-free survival (DFS) (range: 16 to 196 months, mean: 110 months) a primary endpoint.

Results: Improved DFS was noted for LBPC [.25; 95% confidence interval (CI) .08–.76, P = .0149]. When classifying peritumoral lymphoid aggregates, only the presence of well-developed stromal lymphoid aggregates was significantly associated with improved DFS (.11, CI: .01-.94, P = .0440). The absence of intratumoral lymphocytes trended toward decreased DFS (2.54, CI: .94-6.89, P = .0667).

Conclusions: Our findings suggest that morphologic TIL assessment in TNBC can be simplified by evaluating 2 binary parameters: 1) classifying a given tumor as LPBC and 2) the presence of well-developed stromal lymphoid aggregates to predict a given patient's risk of recurrence. Additional independent studies are necessary to validate our findings.

138 Prognostic Impact of the Size of Extracapsular Extension of Axillary Lymph Node Metastases in Breast Cancer: Association with Clinicopathological Features and Outcome

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Background: Extracapsular extension (ECE) of nodal metastasis is associated with a higher risk of mortality and disease recurrence in breast cancer (BC) patients (pts), while the size of ECE in the SLN has been correlated with nodal tumor burden at ALND. However, the prognostic value of the ECE extent remains unclear. Here we examine the association of the ECE size in axillary metastases with clinicopathological criteria and outcome in BC.

Design: Pts diagnosed with node-positive BC from 1994-2014 were selected at a single university hospital. Chart review documented clinicopathological data. Original H&E slides were re-evaluated to determine tumor histological type/grade, presence, size and area of ECE (largest size perpendicular to the node capsule, PECE; size measured transversely to the capsule, TECE; area, AECE). Comparisons were made by extent (<2 mm vs ≥2 mm) and area of ECE.

Results: Our database quest identified 1,238 node-positive BC pts over a 20 year period and 632 were excluded because of missing follow-up, leaving 605 for evaluation (99% female, 1% male). Mean age at diagnosis was 54 yrs (28-90 yrs), median follow-up was 40 mos (1-394 mos), 35% of pts had developed a recurrence by the time of last follow-up and 9% died of the disease. Most tumors were classified as invasive ductal carcinoma (NST, 68%), mainly of grade 2 (43%) or 3 (42%), and the majority of pts was diagnosed at stage II (34%) or III (62%). Mean number of axillary nodes examined/patient was 18 (mean, positive nodes/patient=7) and ECE was observed in 393/605 (65%) cases. When ECE was present, pts had both shorter overall survival (OS) and disease-free survival (DFS; p<0.01). Compared to PECE<2 mm, PECE≥2 mm was associated with the presence of >10 positive nodes at ALND, axillary tumor implants, high tumor grade, disease recurrence and poorer DFS (p=0.02); whereas TECE≥2 mm was correlated with advanced stage, higher number of positive nodes, recurrence, shorter OS and DFS (p<0.01). Further, AECE≥4 mm² was associated with tumor size, higher number of positive nodes and poorer DFS (p<0.01). There was also a correlation between a higher number of ECE foci/patient and tumor size, axillary neoplastic emboli, HER2 overexpression and disease relapse (p<0.01).

Conclusions: Our findings confirm that ECE is associated with worse outcome in BC. Extent, area and number of foci of ECE are correlated with aggressive tumor characteristics, nodal tumor burden, recurrent disease and poorer survival in breast cancer pts.

139 Presence of Cysts in Benign Breast Tissue and the Risk of Subsequent Breast Cancer in African American Women

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Background: Benign breast disease (BBD) is an established risk factor for developing breast cancer (BC) and certain pathologic features in benign breast tissue are more strongly associated with BC risk. Most of the studies evaluating BBD and BC risk have been done in primarily white populations. Our previous work among African American (AA) women with BBD suggested the presence of cysts was associated with increased BC risk. This finding may be unique to AA women as other studies among white populations have not shown this. We sought to further explore the association between breast cysts and BC risk in our expanded AA BBD cohort.

Design: Biopsies from AA women diagnosed with BBD from 1997 to 2009 were examined for 14 benign features, including the presence of cysts, and followed for subsequent BC. The association between cysts and the other pathologic features were compared using chi-square tests, and the risk of developing BC was estimated using logistic regression and summarized with odds ratios (OR) and 95% confidence intervals (95% CI). Results were then stratified by age at biopsy (<40 years versus 40+ years).

Results: A total of 3,360 AA women with BBD have been identified and their benign biopsies reviewed. 190 women have developed a subsequent BC with a mean follow-up time of 11.5 years. Cysts were present on 1,366 (38%) of the biopsies, and were significantly associated with nearly all of the other benign features evaluated (apocrine metaplasia, ductal hyperplasia, calcifications, duct ectasia, fibrodenomas, fibrosis, intraductal papillomas, sclerosing adenosis, columnar alterations, mucocele-like lesions, radial scars, and proliferation with and without atypia, all p<0.001). Adjusting for age and year of biopsy, cysts were associated with a 36% increase in BC risk (OR: 1.36, 95% CI 1.01 – 1.82). When further adjusted for hyperplasia with atypia, the benign feature with the most established and strongest association with breast cancer risk, the risk associated with cysts was attenuated (OR: 1.20, 95% CI 0.88 – 1.64). Stratified results suggest that the risk associated with cysts is stronger for women first diagnosed with BBD under 40 years (OR for <40: 3.24, 95% CI 1.08-9.75, OR for 40+: 1.23, 95% CI 0.91 – 1.68, p-interaction = 0.098).

Conclusions: Among AA women, cysts are highly correlated with other BBD features that incur increased subsequent breast cancer risk, but the risk associated with cysts does not appear to be independent. Understanding the etiology of cyst development and other associated BBD features may provide insight into tumorigenesis.

140 Value and Clinical Utility of Oncotype Dx for Patients with Recurrence Scores of 10 or Less: An Independent Value Study of Tumor Histopathology and Outcomes

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Background: The breast cancer gene expression profile literature rarely details tumor histopathology in their outcome studies, thus, histopathology of breast carcinomas tends to be minimized for risk assessment. In the paper of Sparano et al (Prospective validation of a 21-gene expression assay in breast cancer 2015 NEJM 373(21) 2005-14), detailed histopathology of tumors was not given. The aims of this study are to detail breast tumor histopathology of patients with Oncotype Dx (ODx) Recurrence Scores (RS) of 10 or less and determine if ODx has value or clinical utility in this group.

Design: 459 patients with ODx RS of 10 or less from a dataset of 1860 patients with ODx results were studied. Patients had five years of follow-up and were treated with endocrine therapy alone. Tissue was available to review on 441 patients, and details included (1) type of carcinoma (2) mitotic score (MS), tubule formation, nuclear pleomorphism and Notttingham histologic (NG) grade. (3) Estrogen (ER) and progesterone (PgR) semi-quantitated by Histologic Score (H Score: strong 200-300, moderate 100-199, weak <100). (4) Lymph node status. (5) Overall survival and breast cancer specific survival.

Results: 148 of 441(33.5%) patients had carcinomas of special types, including tubular 22(15.5%), cribriform 15 (10.8%), papillary 17 (11.5%), mucinous 25 (21.6%), lobular 57 (38.5%) and mixed ductal and lobular 3 (2%). All special type tumors had a MS of 1 and were NG1. The remaining 293 tumors were ductal carcinoma no special type (NST), and 261/293 (89%) had a MS of 1/NG2. Of the remaining 32 cases, 10 (11%) had a MS of 2/NG2, 18 cases had mitosis score of 2/NG3 and four cases were MS3/NG3. All patients were lymph node negative. ER was strong in 89.6%, moderate in 10.2% and weak in 0.2%. At 5 year follow-up, 433/441 patients (98%) were alive, 8 were dead, 1 from breast cancer. Five patients had local recurrence and 2 had distant recurrence.
Conclusions: (1) Breast carcinomas of special type have MS1/NG1 and ODx in this group lacks value and clinical utility. (2) NST breast carcinomas that have MS1, NG1/2 and high ER content are very low clinical risk tumors i.e. Luminal A tumors. ODx also lacks value and clinical utility in this setting. (3) Both of these tumor groups have a 5 year breast cancer specific survival of 99.8%. (4) If pathologists guide the use of ODx for this population, 24% of ODx cases could be eliminated at a national cost savings of 207,222,581 USD per year.

141 Breast Cancer Global Tumor Biomarkers: A Quality Assurance Study of Intratumor Heterogeneity

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Background: Biomarkers (BM) are typically performed on core biopsies (CB) of breast tumors, yet comprehensive information on BM heterogeneity is lacking. This study is to survey BM intra-tumor heterogeneity (ITH) by comparing BM from CB and the examination of entire breast tumors from surgical excisions (SE).
Design: CB and SE of entire tumors 3.0cm or less, comprising from 1 to 22 blocks per case, were all examined by IHC for ER/PR/HER2/Ki67 or HER2 ISH for in situ (IS) and invasive (IV) disease. Clones ER (SP1), PR (1E2) HER2(4B5) Ki67 (30-9) and dual ISH for HER2 were used. Whole slides were semi-quantitated for ER/PR using H-Score (HS) (0-300), HER2 was interpreted using 2013 ASCO/CAP guidelines, and Ki67 proliferation index (PI) was estimated as whole slide and hot spots. ITH was measured between CB and SE, and by comparing the mean (global score, GS) of all tissue blocks to individual tissue blocks.
Results: Of 101 consecutive patients, mean age was 60 years: 81% ER+PR+, 12% ER+PR-, 7% ER-PR-. 74/101(73%) had ER HS ≥200, 15/101(15%) ER HS100-199, 12/101(12%) ER HS <100. Categorical results for ER were all in agreement for CB vs SE. Coefficient of variation (CV) for ER HS showed up to a 5-fold inter-block variation, and PR CV was significantly higher indicating greater inter-block heterogeneity . Higher CVs were seen with decreasing ER HS. ER HS in-situ correlated with ER HS invasive disease. Ki67 hotspots were identified in 57% of SE, but in only 13% of CB. HER2 was positive in 13/101cases (13%). 4/13 (30%) of HER2 cases were heterogenous 2+ CB but 3+ SE, two cases were IHC 2+/ISH amplified and 7/13 (54%) were both CB and SE IHC 3+.
Conclusions: (1) ITH of ER is most common when ER content decreases (2) PR ITH is more common than ER (3) ER HS of in-situ disease correlates with ER HS of invasive tumor. (4) Ki67 CB correlates with SE, but hot spots are seen in 57% of SE compared to 13% of CB. (4) 30% of HER+ cases were heterogenous. (5) BM ITH may affect Her2 results and potentially, gene expression profile test results.

142 High Discordance of Separate Assessments of Nottingham Nuclear Pleomorphism Score Is a Strength; Empirical Proof of Why the Kappa Statistic Can Be Misleading

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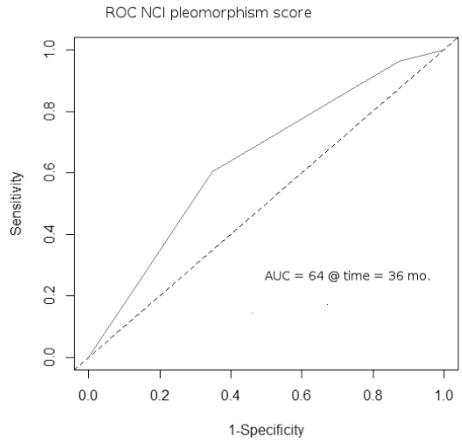
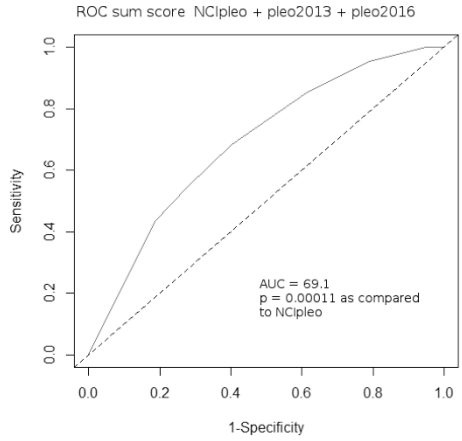
Background: Separate assessments of Nottingham nuclear pleomorphism score (pleo) provided the means to generate discordant assessments. This allowed for study of discordance as it relates to patient survival.
Design: Three separate assessments of pleo were performed on each of 1085 breast cancers obtained from USA Natl. Cancer Inst. (NCI). Two of the readings were intra-observer and performed in years 2013 and 2016 (3 yr interval allowing for memory "wash-out"). On each cancer, pleo had also been assigned by NCI (NCIpleo). Statistical analysis included: kappa statistic (KS), survival, and time-dependent receiver operator curves.
Results: By log-rank each separate reading of pleo corresponded to significant survival stratification (all p<0.001) -- all readings were relevant. KS were: intra-observer pleo2013 vs pleo2016 = 0.49; inter-observer pleo2013/pleoNCI=0.34 and pleo2016/pleoNCI=0.32. Exact agreement among all readings was only 42%, yet 1% high/low discordance. Area under curve (AUC) of NCIpleo at 36mo survival endpoint=64 (fig 1). After pleo2013 score and NCIpleo were summed together (possible sum scores 2-6), AUC significantly increased to 67.6 (p = 0.003). Note that sum scores 3&5 could only be reached via discordant readings. Upon further adding pleo2016 (yielding possible sum scores 3-9) AUC again increased to 69.1 (fig2). As point of reference, AUC of Nottingham score=68.7.





Conclusions: Contrary to popular belief, a strength of pleo is high discordance. Since hi/low discordance is rare, discordant assessments are not randomly distributed, but are points along a scale. This leads to an improved description of the underlying continuous spectrum of patient survival. A low KS can be a promising number. Shown here has been an example of how quantifying redundancy via KS, without corresponding study of relevance, would have been insufficient analysis.

143 Nucleolar Prominence as the Only Morphological Attribute Used in Determining Nuclear Pleomorphism: Evaluation Based on Tissue Microarray Analysis

Leslie Dalton. South Austin Medical Center, Austin, TX.

Background: Assessment of nuclear pleomorphism is regarded as the most troubled of Nottingham components, and is determined by a molding of several morphological attributes to include nucleolar prominence (NP). NP is strongly correlated with tumor proliferation and measuring proliferation is considered the most important factor in predicting patient survival. Used here was a tissue micorarray (TMA) platform to evaluate importance of NP.
Design: 1085 tissue microarray samples (TMA) of breast cancer were obtained from USA Natl Cancer Inst (NCI). The NCI had designed case selection such that a promising biomarker would be associated with strong statistical power. Three methods for defining NP were evaluated. First was visibility of NP at low microscopic power (LMP) with: 100X LMP score 3; 200X LMP score 2; absent/inconspicuous nucleoli score 1. Second method was an eyepiece reticle measurement of NP (RNP) with: >2.5 micron nucleoli score 3; RNP>1.2 microns score 2; RNP<1.2 score 1. The third method was a subjective approach (SNP) which was guided by Helpap's description. Score 3 SNP had nuclei with at least 2, or an extreme example of, multiple nucleoli, placement of nucleoli along nuclear membranes, nucleolar dysmorphism, and nucleolar enlargement. SNP score 1 had tumor nuclei with absent or inconspicuous nucleoli. SNP score 2 was in-between. For each cancer, the NCI had provided data on Nottingham grade to include mitotic score (MS), and a "traditional" pleomorphism score.
Results: The table below supplies the pertinent results. Given are the p-values corresponding to significance of survival stratification (log-rank method) for each ternary attribute in the stage 1, stage 2, and stage 3 TMAs.

Attribute	Stage1	Stage 2	Stage 3
NCI mitotic score	0.03	0.0006	0.006
NCI pleomorphism score	0.32	0.003	0.02
NCI Nottingham grade	0.28	0.0004	0.0001
Eyepiece reticle measure of nucleolar prominence	0.82	0.0003	0.002
Low microscopic power nucleolar prominence	0.55	0.0002	0.01
Subjective nucleolar prominence	0.001	0.001	0.003

Conclusions: Across all stages, SNP rivaled MS as a "promising" biomarker, and both LMP & RNP were significant in stages 2 and 3. Calling upon Occam's razor, it would be simpler to assess nuclear pleomorphism based on a single attribute rather than an amalgam of several. Technically, a validation study would be required to formally adopt a NP only simplification. In the meantime, do not ignore NP.

144 Reporting the Size of Invasive Mammary Carcinoma in Ultrasound-Guided Core Needle Biopsy Is Crucial for Accurate Final Pathologic Staging

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Background: Second to lymph nodes status, the tumor size and the pathologic T (pT) stage is a strong prognostic predictor in invasive mammary carcinoma (IMC). The purpose of this study is to evaluate the correlation between IMC size, as determined by ultrasound (US), and the size measured on US-guided core needle biopsy (CNB) and subsequent follow-up excision (FUE).

Design: The pathologic and clinical data of 223 consecutive (from 10/2013-8/2016) IMCs that were examined and biopsied under US guidance for mass or architectural distortion were evaluated. In cases with multiple invasive foci, the largest focus was evaluated. Cases which received neoadjuvant chemotherapy were excluded.

Results: Tumor size was classified into 4 groups (≤5, >5-≤10, >10-≤20 and >20 mm) and collectively categorized into small (pT1a and pT1b; ≤10mm) and large (pT1c+; >10mm) groups. Needles of 14 and 12-gauge were used in 120 and 103 cases, respectively. There was an average of 5 cores per case (range of 1-15). Tumor sizes determined by US, CNB and FUE were 4-81mm, 1.1-18mm and 1.5-100 mm, respectively. The concordance between the size determined by US and the final size (largest size either by CNB or FUE) was 69% (K 0.86, CI, 0.43-0.61; r = 0.66). The concordance, overestimation and underestimation rates of (≤10mm) vs. (>10mm) tumors by US in relation to the final size were 80%, 15% and 22%, respectively (K 0.85, CI, 0.40-0.59) with no significant difference in needle gauge or number of cores submitted. In 18 of 223 (8%) cases, the tumor size was greater in the CNBs, resulting in upstaging of 7 cases from pT1a to pT1b. The average size of these 18 cases by US was 7.2 mm (range of 5-9 mm) and the average size difference between the CNB and the FUE was 1.6 mm (range of 0.5-4.5 mm). All the 18 cases were IMC of no special type. The concordance rate in identifying the size group by the CNB in relation to FUE for cases where CNB size is ≤ FUE (n=205) was 21%, while the concordance rate in categorizing small vs. large tumors by the CNB was 90/205 (44%) (K 0.14, CI, 0.03-0.24).

Conclusions: US successfully identified large (>10 mm) and small (≤10 mm) sized IMCs in 80% of cases. We found that the linear extent of IMC on CNB was larger than on FUE in a significant proportion of cases (8%), resulting in pT upstaging from the FUE tumor size. This finding further highlights the importance of reporting the maximum linear extent of IMC upfront on the CNBs, especially in ≤10 mm tumors as reported by US.

145 Can Features Evaluated in the Routine Pathologic Assessment of Invasive Breast Cancer Be Used to Predict the MammaPrint Clinical Risk Assessment Category?

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Background: MammaPrint is a 70-gene signature used to predict the risk of recurrence for early stage invasive breast cancer (IBC) . The results are dichotomized into a low risk and a high risk category with a 10% and 29% recurrence risk at 10 years respectively. The gene signature includes markers of proliferation and downstream targets of hormone receptor pathway, both of which are assessed, in part, by routine pathologic evalution of IBC. We have previously demonstrated that a combination of PR expression levels (> or< 10%) as well as mitotic count score can be used to predict the Oncotype DX Recurrence Score. Our objective in the current study was to determine the relationship, if any, between PR expression and/or mitotic count score and MammaPrint risk categorization results.

Design: We studied 60 cases of ER positive IBC where MammaPrint assessment was performed on formalin fixed tissue . Grading was performed using the Nottingham Grading System . For PR expression the % of positive tumor nuclei was reported and dichotomized into <=10% and >10%. Patient age, tumor size, and lymph node status were abstracted from the pathology reports

Results: Thirty two cases was classified as MammaPrint high risk and 28 as MammaPrint low risk . The mean age was 57.3 years for high risk cases (range 30-77 years) and 63.5 years for low risk cases (range 40-83 years). The median tumor size was 17 mm for high risk cases (range 6-75mm) and 18 mm for low risk cases (range 5-200mm). Twelve high risk cases had positive axillary lymph nodes and 13 low risk cases had positive axillary lymph nodes. Results of MammaPrint risk category as relates to PR expression and mitotic count score are shown in the table:

	MammaPrint High Risk	MammaPrint Low Risk	p value
PR<=10%	18	5	.0033
PR>10%	14	23	
Mitotic Count Score >=2	19	2	.0001
Mitotic Count Score=1	13	26	

There were 9 patients with both PR<=10% and mitotic count score >=2 and 8 had a MammaPrint high risk assessment result. There were 6 patients with mitotic count score of 3 and all 6 had a high MammaPrint Risk assessment result.

Conclusions: We have demonstrated a highly significant association between mitotic count score >=2 and PR <=10% in IBC and MammaPrint high risk result in our cohort.

146 PD-L1 Expression and Intratumoral Heterogeneity Across Breast Cancer Subtypes: An Assessment of 245 Primary and 40 Metastatic Tumors

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Background: Tumor expression of programed death ligand (PD-L1) has been associated with immune evasion in a subset of high-grade, triple-negative breast carcinomas (TNBC) and may mark these cancers as susceptible to PD-1/PD-L1 inhibitor therapies. Little is known about PD-L1 expression in tumor and peritumoral immune cells across the full range of breast cancer subtypes, nor has reliability of core biopsy in determining PD-L1 status been established. It is also unknown whether PD-L1 is acquired as a mechanism of metastatic spread. We address PD-L1 tumoral and immune expression in breast cancers with a range of histomorphologies and investigate intratumoral PD-L1 heterogeneity as well as fidelity across breast cancer primaries and metastases.

Design: Cases were evaluated on tissue microarrays (TMAs) containing 245 invasive primary breast cancers, 20 nodal metastases, and 20 distant metastases (4 x 0.6 mm replicate cores/case). Membranous immunohistochemical staining for PD-L1 was scored by extent in the tumor cells and immune compartment.

Results: Tumor PD-L1 staining was seen in 12% of primaries including 32% of TNBC. High proportions of staining were seen in cancers with medullary (54%), apocrine (27%), neuroendocrine (100%), and metaplastic features (40%). However, diffuse (>50%) staining was relatively rare (2% of all cancers and 5% of TNBC). Immune staining was seen in 29% of all primaries and 61% of TNBC. Tumoral PD-L1 was conserved in 94% of matched primary/metastasis pairs while immune staining showed fidelity in 71%; the remaining cases acquired PD-L1 immune cell expression in the metastasis. Only half of PD-L1 positive tumors showed concordance across all 4 TMA cores.

Conclusions: PD-L1 expression is prevalent among high-grade breast cancers including those with medullary, apocrine, neuroendocrine, and metaplastic morphologies. Tumoral PD-L1 expression shows fidelity between primary and metastatic sites in treatment-naïve cancers; however, acquisition of immune PD-L1 staining at sites of metastasis is not uncommon. There is considerable intratumoral heterogeneity in PD-L1 expression, undermining the suitability of core biopsy in the determination of PD-L1 status. Clinical trials are needed to establish staining thresholds required for response to PD-1/PD-L1 inhibitors; if diffuse tumor cell staining is required, only a small proportion of breast cancers will be appropriate candidates.

147 *HER2* Somatic Mutation Analysis in Breast Cancer: Correlation with Clinicopathologic Features

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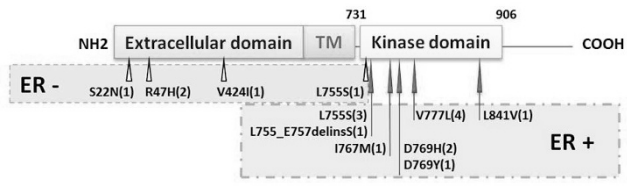
Background: *HER2* mutations have recently been reported in approximately 2% of breast cancers (BCs). Regardless of HER2 overexpression or amplification status, BCs with *HER2* mutations may respond to HER2-targeted therapy. Therefore, the identification of *HER2*-mutated BCs has clinical significance. However, the histopathological features and biomarker profile of *HER2*-mutated BC have not been studied previously.

Design: We retrospectively screened patients with metastatic BC for whom molecular profiles were performed by next generation sequencing (NGS) from 2012-2015 and identified 18 patients with *HER2* mutations. Clinicopathologic features were evaluated for their correlation with NGS results.

Results: Mutations were found on NGS-based panels including Ion AmpSeq Cancer Hotspot (v1 and v2; n=13), Oncomine (n=1), FoundationOne (n=3), and Guardant360 (n=1) panels. *HER2* mutations were identified in both the tyrosine kinase (n=14) and extracellular (n=4) domains.



Of the 14 cases with tyrosine kinase domain mutations, 13 were ER-positive, whereas the 4 cases with extracellular domain mutations were exclusively ER-negative (p=0.002). *HER2* amplification was present in 7 of 18 cases (6/14 mutations in the kinase domain and 1/4 mutations in the extracellular domain). Regarding the histologic type of the primary BC, 13 patients had invasive ductal carcinoma, 1 had ductal carcinoma in situ, and 4 had non-classic invasive lobular carcinoma. *HER2* mutations at L755 site were seen predominantly in the invasive lobular carcinomas.

HER2 mutation	NON-classical Lobular	Ductal	P value
L755	4	1	
Non-L755	0	13	P=0.002

Of 14 patients with tyrosine kinase domain mutations, 11 had bone metastasis, whereas no patients with *HER2* extracellular domain mutations had bone metastasis (p=0.01). The same mutations were identified in matched primary and metastatic tumors in 2 of 2 patients.

Conclusions: Multiple novel *HER2* mutations in the extracellular domain have been identified in BC, and 39% of BC patients with *HER2* mutations have *HER2* amplification. Specific mutation sites may be involved in the pathogenesis of non-classic invasive lobular carcinoma, and identification of these mutations in primary BC may possibly predict the development of site-specific metastases.

148 Atypical Hyperplasia Diagnosed on Breast Core Biopsy: Long-Term Follow-Up of Patients Not Upgraded to Carcinoma on Excision

Alana R Donaldson, Caitlin McCarthy, Valentina Avkshtol, Shazia Goraya, Holly J Pederson, Charles D Sturgis, Stephen R Grobmyer, Benjamin C Calhoun. Cleveland Clinic, Cleveland, OH.

Background: Risk estimates for atypical hyperplasia (AH) are largely based on case-control studies of open biopsies from the pre-core biopsy era. The goal of this study was to correlate core biopsy (CB) findings with subsequent breast cancer (BC) incidence in patients with AH who were not upgraded on excision.

Design: With IRB approval, a CoPath search identified 394 cases of atypical ductal hyperplasia (ADH) and atypical lobular hyperplasia/lobular carcinoma in situ (ALH/LCIS) on CB from 1995-2010. An excision pathology report was available for 349 (89%). Upgrades were defined as invasive mammary carcinoma (IMC), ductal carcinoma in situ (DCIS) or pleomorphic LCIS on excision. The 266 (67%) cases that were not upgraded were selected for detailed review. *P* values were calculated using a χ^2 test.

Results: The mean age was 57 years with a median follow-up of 87 months (range 2 - 236). Slides were available for 217 (82%) cases and diagnoses of ADH (61%), ALH/LCIS (33%), or ADH and ALH/LCIS (6%) were confirmed. There were 54/266 (20%) patients with a history of prior or concurrent BC who were excluded from further analysis. Of the remaining 212, 18 (8.5%) developed a subsequent BC: 13 IMC, 4 DCIS, and 1 of unknown type with a median time to diagnosis of 69 months (range 15 - 155 months). Of the 13 IMC, 8 (62%) were ipsilateral, 9 (69%) Stage I, 11 (85%) estrogen receptor (ER)-positive, and 1 (8%) HER2 amplified. The CB diagnoses for patients with subsequent carcinoma were ADH (10/18) and ALH/LCIS (8/18). The distribution of CB with 1, 2, or ≥3 foci of AH was not statistically different between the patients who did and did not develop BC ($P = 0.25$). Of the patients with subsequent BC, 2 (11%) received chemopreventive therapy, a lower proportion compared to those who did not develop cancer (18%).

Conclusions: In a group of patients with no personal history of BC and a CB diagnosis of AH that was not upgraded to carcinoma on excision, 8.5% developed BC after a median of 69 months. The majority of the tumors that developed were ipsilateral, Stage I, ER-positive invasive carcinomas. The data support close clinical and radiologic follow-up for more than 5 years in this patient population.

149 Atypical Hyperplasia of the Breast on Core Biopsy: Histologic Features Associated with Increased Risk of Upgrade to Carcinoma on Excision

Alana R Donaldson, Caitlin McCarthy, Valentina Avkshtol, Shazia Goraya, Holly J Pederson, Charles D Sturgis, Stephen R Grobmyer, Benjamin C Calhoun. Cleveland Clinic, Cleveland, OH.

Background: The risk of upgrade to carcinoma on excision drives the management of patients with atypical hyperplasia (AH) diagnosed on core biopsy (CB). The goal of this study was to identify CB findings that may correlate with increased risk of upgrade on excision.

Design: With IRB approval, a CoPath search identified 394 cases of atypical ductal hyperplasia (ADH) and atypical lobular hyperplasia/lobular carcinoma in situ (ALH/LCIS) on CB from 1995-2010. An excision pathology report was available for 349 (89%). Upgrades were defined as invasive mammary carcinoma (IMC), ductal carcinoma in situ (DCIS) or pleomorphic LCIS on excision. Slides were available for 105/128 (82%) upgraded cases. The number of AH foci and whether a case was reported as "cannot exclude" or "suspicious for" DCIS were recorded. The majority of the CB suspicious for DCIS either had a low-nuclear grade intraductal proliferation partially involving ducts and lobular units or cytologic features approaching nuclear grade 2 DCIS that were limited to 1 or 2 duct spaces or an area <2 mm. *P* values were calculated using χ^2 and Fisher exact tests.

Results: Of the 394 cases, 128 (32%) were upgraded to carcinoma on excision. The upgrade diagnoses were 46/128 (36%) IMC, 78/128 (61%) DCIS only, 2/128 (1.5%) DCIS with microinvasion, and 1/128 (<1%) pleomorphic LCIS. For cases upgraded to DCIS, 45% were grade 1, 35% grade 2, 15% grade 3, and 5% unknown. The CB diagnoses for upgraded cases were ADH (80%), ALH/LCIS (13%), or ADH and ALH/LCIS (6%). In contrast, the CB diagnoses for cases not upgraded were ADH (60%), ALH/LCIS (33%), or ADH and ALH/LCIS (6%) ($P < 0.001$). Cases suspicious for DCIS had an upgrade rate of 65% (60/92), which was significantly higher than the overall upgrade rate (32%) ($P < 0.001$). Core biopsies with ≥3 atypical foci were more likely to be upgraded (49%) than those with <3 foci (26%) (P<0.001).

Conclusions: There were more cases with ADH and fewer with ALH/LCIS in the group of CB that were upgraded to carcinoma on excision. Cases with ≥3 foci of AH and cases of ADH reported as "cannot exclude" or "suspicious for" DCIS were more likely to be upgraded. The extent of AH and suspicion for DCIS may help identify patients at increased risk for upgrade to carcinoma on excision.

150 Clinical Interplay Between Deubiquitinase 3 (Dub 3) and Snail1 in Breast Cancer

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Background: Expression of Snail1, an important transcriptional factor, correlates with invasive breast cancer tumor grade and nodal metastasis and predicts a poor patient outcome. Snail1 is a labile protein subject to the ubiquitination degradation pathway. Our previous research identified that Dub3 functions as a Snail1 deubiquitinase and promotes migration and invasion through Snail1 stabilization both *in vitro* and *in vivo*; however, whether Dub3 is associated with Snail1 in clinical breast cancer samples is unknown. The goal of this research is to characterize the relationship between Dub3 and Snail1 expression in human breast cancer.

Design: A human breast cancer tissue microarray (TMA) was designed and constructed for evaluation of 334 breast cancers, including 110 cases of luminal, 59 cases of HER2-overexpression, and 165 cases of triple negative breast cancer (TNBC). A total of 1002 tissue cores (triplet core specimens for each case) were immunostained using antibodies against Dub3 or Snail1. A composite staining index was based on the product staining intensity (range 0-3+) and staining percentage of tumor cells in each core. The results were ranked in three expression levels: high (3+), low (2+), and negative (0-1+). The data was analyzed by ANOVA. We also searched the TCGA and Finak gene expression databases to identify surviving patients without distant metastases, and stratified them based on Dub3 expression level (low and high).

Results: The staining of Dub3 and Snail is absent or very weak in normal breast tissue. Both Snail1 and Dub3 are highly expressed in the TNBC group but not in the non TNBC groups (P=0.001, R=0.643 and P=0.010, R=0.379, respectively). Most importantly, Snail1 expression is positively correlated with Dub3 expression in TNBC (90/165 cases, P=0.010, R=0.488) but not in the other groups (17/166 cases). In addition, Kaplan-Meier analysis showed that individuals with high Dub3 expression had a significantly higher probability of developing distant metastases and a reduced disease-free survival interval (P=0.037, TCGA database and P=0.011, Finak database).

Conclusions: Intensity and distribution of Dub3 expression positively correlated with Snail1 in TNBC. Outcome data suggests Dub3 expression may represent an important prognostic indicator for breast cancer patients in the clinical setting.

151 Clinicopathologic Findings in Female to Male Gender Reassignment Surgery

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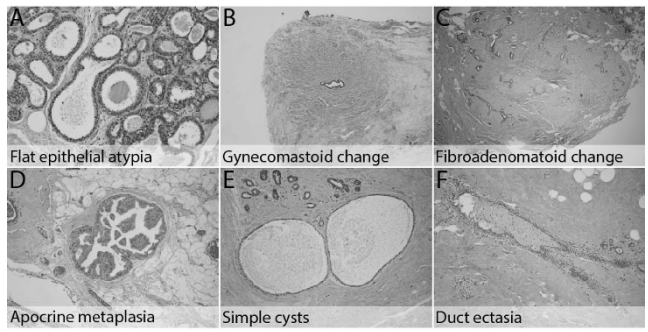
Background: Gender dysphoria is a condition wherein an individual identifies as the opposite gender. Management of patients seeking female to male (FTM) transition may include surgical intervention including mastectomy and hormonal therapy. We describe the pathologic findings in FTM patients undergoing surgical excision of breast tissue.

Design: We retrospectively evaluated clinical and histologic findings of 68 cases (67 patients, 1 with re-excision) of FTM surgical excision of breast tissue.

Results: Mean age at surgery was 31.3 yrs (range 19-57). 51 of 67 (76.1%) patients had androgen therapy, with mean duration of 1.72 yrs.

Specimens weight was recorded in 91.2% (62/68), with mean weight of 550 g. A median of 6 (range 2-20) tissue pieces were submitted in a median of 2 (range 2-16) cassettes per case. Mean gross fibrous density was 26.3%. Gross lesions were identified in 19.1% (13/68).

Terminal duct lobular units (TDLUs) were absent in 11.8% (8/68), rare (<3 TDLUs) in 27.9% (19/68) and frequent (>3 TDLUs) in 60.3% (41/68) of cases. Diagnostic findings were present in 75% (51/68) of cases. Fibrocystic changes (27/68; 39.7%) were most frequent, consisting of simple cysts (22/68, 32.4%), apocrine metaplasia (16/68, 23.5%), adenosis (5/68;7.4%) and usual ductal hyperplasia (3/68; 4.4%). Also seen were gynecomastoid change (22/68, 32.4%), duct ectasia (12/68, 17.6%), fibroadenomatoid change (11/68, 16.2%), lactational change (2/68; 2.9%) and intraductal papilloma (1/68; 1.5%). One case had a high risk lesion of flat epithelial atypia (1/68, 1.5%). Representative histologic lesions are shown in Figure 1.



Conclusions: Evaluation of FTM breast specimens provides insight into androgen-stimulated breast tissue in a unique patient population. The most common histologic findings are non-proliferative fibrocystic changes; however, risk lesions are also infrequently identified. Additionally, the frequent finding of gynecomastoid change is unusual considering its otherwise rare existence in female patients. Our findings support continued gross and histologic evaluation of specimens from patients undergoing FTM gender reassignment.

152 The Ki67 Index as a Prognostic Marker in Primary Hormone Receptor Positive/ Her2 Negative Luminal Breast Carcinoma

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Background: The 12th St Gallen International Breast Cancer Conference, reclassified the molecular subtypes of breast carcinoma into classes that correlate with prognosis. Differentiating Luminal A from Luminal B/HER2 negative breast cancers carries important therapeutic implications, and is based primarily on the proliferation status. However, the Ki67 cut-off for differentiating these two categories changed over time.

Design: We analyzed a retrospective cohort of 112 HR+/HER2 negative luminal breast carcinomas using a ki67 cut-off of <14% to define the luminal A cases. We aimed to assess the prognostic significance of this Ki67 cut-off and also to test the optimal Ki67 cut-off to stratify cases based on recurrence and disease-free interval (DFI) at 2 years

follow up. The classification and regression trees-guided (CART-guided) methods were utilized to determine the independent predictors of tumor recurrence and to determine the optimum Ki-67 cut-off.
Results: The median Ki67 index differed significantly between luminal A (Mdn=5% , IQR=7.5%) and luminal B tumors (Mdn=77%, IQR=30%). Luminal A tumors (ki-index <14%) exhibited significantly longer DFI than luminal B tumors. Using the CART determined cut-off value-, cases were categorized into two groups; (groups I: ki67 < 21%, II: ki67 ≥ 21%). Group I patients (luminal A and luminal B tumors with low ki67) exhibited a lower incidence of recurrence at a longer DFI compared to group II patients (luminal B tumors with high ki67) irrespective of the nodal and PR status. Among group II patients, negative nodal status was an independent predictor of better outcome, while high PR predicted better outcome only in node positive cases.
Conclusions: We confirm previous reports that the 20% Ki67 cut-off is the best to stratify high-risk patients inHR+/HER2 negative luminal breast cancers. Ki67 represents a single independent predictor of better outcome among HR+/HER2 negative tumors with low ki67 index, and may therefore be used to identify high risk patients among luminal B HER negative tumors. High PR reflects better outcome only in node positive HR+/HER2 negative tumors with high ki67 index. Studies on larger cohorts are recommended to establish standardized optimal ki67 cut-off point before being used to select HR+/HER2 negative luminal patients who might benefit from adjuvant chemotherapy.

153 Should Women Assessed at Screening and Diagnosed with ADH on Core Needle Biopsy Be Included in Trials of Active Surveillance for Low Risk DCIS?

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Background: A needle core biopsy (NCB) diagnosis of Atypical Ductal Hyperplasia (ADH) is an indication for open biopsy (OBx). Meanwhile, two randomized trials of active surveillance are underway for low risk DCIS. This leads to the paradoxical situation of women with ADH having surgery, while those with low grade DCIS are observed. If the malignancies diagnosed after surgery for ADH are predominantly low risk DCIS, the inclusion criteria of DCIS surveillance trials may be extended to ADH, a more common diagnosis at screening than LG DCIS.
Design: In this 10-year prospective observational study at our screening Program, women diagnosed with ADH on NCB were included. We retrieved their surgical pathology data and calculated:
1. The proportion and grades of DCIS and invasive cancers diagnosed at OBx
2. The histologic extent of the malignancies at surgery
3. The biomarker profile and nodal status of any invasive cancers
4. Extrapolation of the above to simulate a policy of active surveillance for women with screen detected ADH

Results: Between Jan 05 to Dec 14, 114 women, mean age 59.0 yrs, were included. Surgical pathology, available in 110 (96.5%), confirmed malignancy in 46 (40.4%), all showing DCIS and accompanied by invasive carcinoma in 9 (8.2%) women. In the pure DCIS cohort the DCIS was low grade in 15, intermediate nuclear grade without necrosis in 11, intermediate nuclear grade with necrosis in 5 and high grade in 6 cases. Altogether, 20 (18.2%) women had invasive cancer, high grade DCIS or necrotizing DCIS. The mean DCIS extent was 19.8mm, range 2-110mm. 32 (29.1%) women required further breast surgery after OBx, including mastectomy in 12 (10.9%). Only 1 of 9 invasive cancers was grade 1, 3 were multifocal, all were ≤8mm, node negative and ER positive but 2 were HER2 amplified.
Conclusions: If active surveillance is adopted for screen detected ADH diagnosed on NCB, 59.6% of women will avoid unnecessary surgery and a further 23.6% would meet eligibility criteria for DCIS surveillance trials. However, 18.2% of women will have undiagnosed invasive breast cancer or extensive non-low risk DCIS. Since ADH and low grade DCIS represent a morphologic continuum, particularly on NCB, these findings likely simulate the disease patterns in women currently being enrolled in DCIS surveillance trials. Biological and genomic risk stratificatoin markers of ADH subtypes would be of value.

154 Differentially Expressed miRNA in the Progression of Infiltrating Ductal Breast Carcinoma

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Background: Relatively little is known of the biological determinants of lymph node metastases from infiltrating ductal carcinoma (IDC) and their mechanistic relationship with the ability to colonize distant organs is disputed. Recently, an increasingly important role is being attributed to microRNAs (miRNAs) as gene expression modulators in normal and cancer cells. To better understand the molecular basis of lymph node involvement in association with IDC, we have explored the differential expression of microRNAs in normal, in situ, invasive and metastatic stages of breast carcinomas.
Design: Samples from normal breast, primary node-negative and node-positive IDC, their matched lymph node metastases, in situ carcinomas and distant metastases, were analysed for differential miRNA expression by hybridization on a 1000-microRNA Agilent microarray platform. Levels of the most significant microRNAs were quantified by qRT-PCR on samples from primary tumors, lymph node metastases and distant metastases.
Results: Several microRNAs were significantly upregulated in the transition from normal breast tissues to primary metastatic tumors, including miR200a, miR-200b and miR-429. Another set of miRNAs, including miR-181a, miR-181b, miR- 210 or miR-7, was upregulated in distant metastases relative to primary tumors. Quantitative PCR confirmed that miR-200b was significantly upregulated from non-metastatic to metastatic primary tumors, miR-7, miR-210, miR-181a and miR-181b were up-regulated in distant metastases relative to node-negative tumors and miR200a, miR-200b and miR-429 in lymph node metastases compared to their primary tumors.
Conclusions: MicroRNAs may be potential biomarkers of metastatic status in invasive ductal carcinoma of the breast. Among them, the miR-200 family, modulators of the epithelial-mesenchymal transition among other functions, may play a role as drivers of aggressiveness through regional and distant dissemination.
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155 High VANGL2 Expression Is Associated with Basal-Like Phenotype in Axillary Node-Negative Breast Cancer

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Background: Vang-like 2 (VANGL2) is a component of the non-canonical Wnt/planar cell polarity pathway that is important for collective cell migration during embryonic development. VANGL2 overexpression is associated with increased breast tumor cell proliferation, increased migration, and decreased metastasis-free survival in breast cancer patients. The aim of this study was to determine if VANGL2 expression was associated with poor prognostic characteristics and decreased disease-free survival (DFS) in patients with axillary node-negative (ANN) breast cancer.
Design: Immunohistochemistry was performed for VANGL2, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), Ki67, and p53 on tissue microarrays generated from 668 prospectively ascertained ANN breast tumors. Tumors were subdivided into the major molecular subgroups (Luminal A, Luminal B, HER2+, basal-like). Luminal subtypes were defined using Ki67, p53 and PR expression. VANGL2 expression was divided into three groups (low, moderate, high) based on Allred score. Descriptive baseline analyses compared frequency distributions of clinicopathologic factors among the three groups. DFS analyses were conducted for association of VANGL2 expression with risk of distant recurrence within the whole group and molecular subgroups by the log-rank test with Kaplan-Meier survival curves.
Results: High VANGL2 expression was significantly associated with poor prognostic features including high histological grade (P=0.0368), ER negativity (P=0.0195), and with the basal-like breast cancer phenotype (P<0.0001). Although the association between high VANGL2 expression and decreased DFS did not reach statistical significance, a trend was observed in both whole group and basal-like subgroup analyses.
Conclusions: The observed association between VANGL2 expression and aggressive tumor characteristics may indicate a functional role for the protein in breast cancer development and progression.

156 Mammary and Extra-Mammary Paget's Disease- An Institutional Experience

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Background: Mammary Paget's disease (MP) and extra-mammary Paget's disease (EMP) are rare with MP constituting 1-4.3% of all breast cancers while data on incidence of EMP is limited. Both diseases are associated with underlying intraductal carcinoma (DCIS) or invasive carcinomas (IC) and clinically mimic inflammatory or infective diseases and hence often diagnosed late. The common morphologic differential diagnoses include malignant melanoma and squamous carcinoma. The aim of this study was to explore institutional incidence of MP and EMP and elucidate their clinicopathologic characteristics.
Design: After institutional review board approval, a search in pathology electronic database was done between 1995 and 2016. Clinicopathologic characteristics such as age, gender, anatomic site, underlying pathologic process, and clinical outcomes were recorded.
Results: Total of 112 cases of Paget's disease were identified [84 (75%) MP, 28 (25%) EMP]. There were 97/112 (86.6%) females and 15/112 (13.4%) males with age range between 33 to 90 years (median=Female: 66 years, Male:70 years). 81/97 (83.5%) females had MP and 16/97 (16.5%) EMP. 3/15 (20%) males had MP and 12/15 EMP (80%). EMP sites included vulva (14); inner thigh (1), perineum (1) in females and scrotum (5); anus (1); perianal region (2), anal verge (1), axilla (1); penis (1); groin (1) in males. 39/84 (46%) MP were associated with invasive ductal carcinoma; 1/84 (1.2%) with mucinous adenocarcinoma and 1/84 with metaplastic carcinoma. The underlying histological diagnoses for EMP included adenocarcinoma involving vulva (1); basal cell carcinoma in perianal mass (1); colonic adenocarcinoma(1); poorly differentiated carcinoma in labia majora (1); adenocarcinoma involving glans penis and urinary bladder (1).
Conclusions: Paget's disease was observed in older females with high frequency of MP while older males had frequent EMP. Vulvar EMP was the most frequent site in females and while scrotum in males. Most MP and EMP had underlying invasive adenocarcinoma.

157 "Is Estrogen Receptor Positive (ER+) Progesterone Receptor Negative (PR-) Invasive Lobular Carcinoma a Distinct Clinicopathologic Subset?"

Ramya Gadde, Dhananjay Chitale. Henry Ford Hospital, Detroit, MI.
Background: Invasive lobular breast cancer (ILC), the second most common histologic subtype of breast cancer (5% to 15%), characteristically presents in small, discohesive epithelial cells, mostly express the estrogen receptor (ER, encoded by the ESR1 gene), lack HER2 (ERBB2) over expression / amplification, and have loss or reduced expression of E- Cadherin protein, a cell adhesion molecule. Even though ILC are distinct from invasive ductal carcinoma (IDC) the current treatment approach

is very similar in both the entities that are predominantly based on hormonal status and stage. Several studies have devoted efforts to differentiate the variants of ILC, but few studies address correlation between ER receptor signaling and progesterone (PR) protein expression and its impact on staging, prognosis. Our aim was to focus on the ER receptor signaling, PR status and to analyze other clinicopathologic variables.
Design: After institutional review board approval, all cases of ILC were retrieved from cancer registry database over 23 years. Electronic medical records were reviewed and clinicopathologic findings including hormonal receptor status, age and clinicopathologic stage were recorded.
Results: Total of 371 cases of ILC were identified where ER, PR results were available. ILC had following ER/PR status: 287/371 (77.3%) ER+/PR+, 58/271 (15.7%) ER+/PR-, 5/271 (1.3%)ER-/PR+, 21/271 (5.7%), ER-/PR-. Patients with ILC age group ranged from 32-94 years (Mean 64.9 years, median 67 years). ER +/PR- patients age range from 45 to 86 years (mean age 69.9 years, median 71.5 years). ER+/PR+ patients age range from 32 to 65 years. (Mean age 63.9 years; median 65 years). 14/53 (26.4%) ER+/PR- were high stage (stage 3,4) vs 34/261 (13%) ER+/PR+ were high stage.
Conclusions: Cases with ER +/PR- tend to occur in older age compared ER+/PR+ cases. ER+/PR- cases appear to have higher stage compared to ER+/PR+.

158 The Inhibitor of DNA Binding Proteins: mRNA Expression Analysis and Prognostic Relevance in Breast Carcinoma

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Background: Inhibitor of DNA binding (ID) proteins family consists of four members (ID1-4). They are functional inhibitors of the basic helix-loop-helix (bHLH) transcriptor factors, with a critical role in developmental and cellular processes. It has been suggested that depending on different cellular contexts, they exert divergent functions, acting as oncoproteins or tumor suppressors. In fact, previous studies have described aberrant expression patterns in several human neoplasias. In breast carcinoma, the pathogenetic role and the clinical significance of IDs proteins are not fully elucidated.
Design: We included 134 non-consecutive patients with breast carcinoma. Tumours were immunohistochemically classified into Luminal A (19%), Luminal B/HER2-negative (21%), Luminal B/HER2-positve (14%), HER2-positive (17%) and Triple-negative/Basal-like (28%). mRNA expression of ID1-4 genes was evaluated by qRT-PCR. Relative changes in gene expression were calculated as the fold-change (FC) by the 2 -ΔΔCt method. PUM1 was the reference gene and normal breast tissue was used as control sample. The expression analysis results were correlated with clinicopathological factors (age, tumor size, histological grade, lymph-vascular invasion, necrosis, lymph-node status and immunophenotype) and patients' outcome. Significant associations were identified using Chi-square and Fisher. Survival was calculated by the Kaplan-Meier method (log-rank test).
Results: mRNA expression levels were predominantly high for ID2 and ID3 (58% and 76%, respectively) and low for ID1 (70%) and ID4 (63%). Higher ID2 levels were seen in tumors of older patients (p=0.013), and for ID3 in those with lymph-vascular invasion (p=0.049), necrosis (p=0.032) and larger tumor size (>20 mm) (p=0.082). In contrast, lower ID1 and ID4 mRNA expression were detected in a subset of Luminal tumors (41%, p=0.038; and 37%; p=0.044; respectively), with negative lymph-node status and grade 3, but only as a trend (all p≤0.13). Overall survival was shorter for patients whose tumors overexpressed ID2 (p=0.021) or ID3 (p=0.0042; Kaplan-Meier; log-rank test).
Conclusions: Our results in a clinical series of breast carcinoma support the potential role of ID1 and ID4 as tumor suppressor genes, specifically in Luminal tumors. Moreover, ID2 and ID3 are plausible poor prognostic markers and therapeutic targets.
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159 mRNA Expression Analysis of Inhibitors of DNA Binding Proteins: Correlation with Epithelial-Mesenchymal Transition and Cancer Stem Cell Markers in Breast Carcinoma

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Background: Inhibitors of DNA binding (ID) proteins are a family of highly conserved transcriptional regulators that are important in both, during developmental processes and in adult tissue homeostasis. Experimental studies suggest their inhibitory role of differentiation and maintenance of self-renewal and multipotency in cancer stem cells (CSC). Furthermore, IDs are thought to increase invasiveness through induction of an epithelial-to-mesenchymal transition (EMT) program at the primary tumor site and the ability to colonize the secondary site through reversal to an epithelial state (mesenchymal-to-epithelial transition, MET). In breast carcinoma, the biological relevance of the IDs in the processes of transformation of dormant normal stem cell to CSC or EMT is not well known.
Design: We included 134 non-consecutive breast carcinomas (19% Luminal A, 21% Luminal B/HER2-negative, 14% Luminal B/HER2-positive, 17% HER2-positive and 28% Triple-negative/Basal-like). We analyzed the mRNA expression of ID1-4, EMT (SNAIL1 and CDH1) and CSC (CD44) biomarkers by qRT-PCR, using TaqMan assays. Relative changes in gene expression were calculated as the fold-change by the $2^{-\Delta\Delta Ct}$ method. PUM1 was the reference gene and normal breast tissue was used as control sample. Biomarkers expression was correlated as well as with breast cancer immunophenotypes. Significant associations were identified using Chi-square and Fisher exact test.
Results: We observed predominantly down-regulation of ID1 (70%), ID4 (63%), SNAIL1 (60%), CDH1 (90%) and CD44 (55%), Conversely, ID2 and ID3 were up-regulated (58% and 76%, respectively). ID1 correlated positively with ID2 and ID4, but negatively with ID3 (all p≤0.000). Similarly, ID2 associated directly with ID3 and ID4 (all p≤0.000). Moreover, expression levels of all IDs correlated with SNAIL1 (p≤0.000), only ID3 with CD44 (p=0.032), but no association was found with CDH1 (p=ns). Higher expression of SNAIL was observed in Triple-negative/Basal-like immunophenotype (p=0.029).
Conclusions: Our results in a series of breast carcinoma show deregulation of IDs1-4, which in turn are differently associated with EMT (SNAIL1) and CSC (CD44) biomarkers, supporting their role in both mechanisms in this neoplasia.
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160 High CD44 Expression in Brain Metastases from Breast Cancer Suggests Role of Cancer Stem Cells

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Background: Approximately 15% of all metastatic breast cancers lead to symptomatic brain metastases. Brain metastases are difficult to treat and have a median survival of less than one year. There is a need to understand this metastatic process to effectively treat disease. Cancer stem cells (CSCs) are a subpopulation of cells within solid tumors that are believed to be responsible for tumor cellular heterogeneity and metastatic potential. The hyaluronic acid receptor CD44 is a commonly used CSC marker. However, little work has been done to investigate CD44 in a clinical setting to date. Of note, there is some evidence that CD44 regulates expression of PD-1/PD-L1 immune pathway and may play a role in tumor immunity. The purpose of this study was to investigate CD44 expression in primary breast tumors and CNS metastases and explore the role CSCs may play.
Design: Tissue microarrays from a well-annotated cohort of breast cancers metastatic to the brain (n=85) and primary breast tumors (n=23) were evaluated for CD44 expression by IHC performed on 5 μm sections. Samples were scored independently by two observers and considered positive if staining was present in greater than 10% of tumor cells. When available, the CD44 staining results were compared between the matched primary and metastases (n=15) and with PD-1 and PD-L1 expression data previously presented on this cohort.
Results: CD44 was expressed in 35% of primary breast tumor tissue (8/23) and in 60% of brain metastases (50/84). When compared with matched primary breast tumors, metastases gained expression in 7 (47%) cases, there was no change in expression in 8 (53%)of the cases. No matched sets showed a loss of expression from primary breast tumor to brain. Notably, though PD-1 was expressed in only 5% of CNS mets, all were CD44+. Metastases which were PD-1 negative displayed CD44 expression in 45/81 (56%) of the cases and lacked expression in 36/81 (39%). PD-L1 was co-expresed with CD44 in 21/84 (25%) of the cases.
Conclusions: Increased expression of CD44 in metastases suggests that CSCs may play a role in processes that underly disease progression and recurrence. Expression of CD44 in all cases with PD-1+ lymphocytes suggests a possible relationship. However, the sample size of PD-1+ tumors is small. As CSCs are innately resistance to conventional chemotherapy, CSCs may prove to be an important therapeutic target. More needs to be understood about CSC marker detection and the role of CSCs in various cancers.

161 Intra-Tumor Genetic Heterogeneity in Metaplastic Breast Carcinomas

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Background: Metaplastic breast carcinoma (MBC) is characterized by squamous and/or mesenchymal differentiation and intra-tumor histologic heterogeneity, which is reflected at the molecular level. Histologically distinct components within MBCs may be of distinct molecular subtypes and display distinct copy number alterations. We sought to investigate whether distinct histologic components within MBCs display different repertoires of somatic genetic alterations and mutational signatures.
Design: We studied 11 MBCs with histologically distinct components (spindle, chondroid, squamous and/ or ductal). Two distinct components of each case and, in two cases, two regions of the same component were separately microdissected. DNA samples extracted from tumor/normal pairs were subjected to whole-exome sequencing. Somatic genetic alterations, the cancer cell fraction, and mutational signatures were identified using state-of-the-art bioinformatics algorithms.
Results: A median of 104 (39-222) non-synonymous somatic mutations per component was identified. In every case, both components harbored identical *TP53* mutations, either coupled withloss of heterozygosity or a second *TP53* mutation. Additional recurrent mutations included those affecting the PI3K pathway (*PIK3CA*, n=2; *PIK3R1*, n=2). Shared somatic mutations between components of each MBC ranged from 34% to 99% (median 84%) of all somatic mutations, whereas shared and clonal (i.e., early event) mutations ranged from 12% to 53% (median 27%). Each component harbored a median of 13.5 (0-145) private non-synonymous mutations, of which 1 (0-92) was clonal. Private non-synonymous mutations in cancer genes included those in *PIK3R1*, *MED12* and *NOTCH1*. The mutational signatures were consistently concordant between distinct components of each MBC. In two cases, two histologically similar regions analyzed were found to be less genetically heterogeneous, with 94% (87%-100%) of shared mutations, and a median of 1.5 (0-3) private non-synonymous mutations per region, of which none was clonal.
Conclusions: MBCs display intra-tumor genetic heterogeneity, which is more overt between histologic distinct components than between distinct regions of the same histology. Our results demonstrate that histologically distinct components of MBCs are clonally related. Distinct somatic mutations, including pathogenic mutations in cancer genes, can be restricted to a specific component within a case, suggesting that histologically distinct components within an MBC may be coincidental with, or underpinned by, distinct somatic genetic alterations.

162 Abstract Withdrawn

163 Loss of *DEEBC* Expression Is Lost Early in Breast Cancer Development

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Background: Accumulating evidence suggests a role of long intergenic RNAs (lincRNAs) in cancers including breast cancer. Our prior studies have identified DEEBC (Decreased early in breast cancer; also called *LINC00478*) as a lncRNA that is decreased in primary ER+ breast cancer with further loss with nodal metastasis. In this study, we sought to compare the expression of this lncRNA in DCIS to assess its role in breast cancer progression.

Design: Quantitative RT-PCR (qRT-PCR) for DEEBC was performed on a series of pure DCIS cases (n=25) and compared with a cohort of matched primary tumors and associated nodal metastasis (n=21 pairs) as well as normal breast tissues from breast reductions specimens (n=10). Briefly, all RNAs were extracted from 10 micron tissue sections after de-paraffinization using the Ambion RecoverAll kit. After quantifying the RNA (Nanodrop), qRT- PCR was performed using the primer-probes pairs specific for the gene using the ABI-7900 TaqMan platform. The results were analyzed using DataAssist software and p-value less than 0.05 was considered significant. Computational analysis was performed using the TCGA cbioportal and RNA-seq data.

Results: Expression of DEEBC was 30% lower in DCIS cases as compared to normal tissue ($P = 0.05$) using the qRT-PCR assay. Further comparison between DCIS and invasive carcinoma (primary and nodal metastases) showed a trend for the decrease to be greater in invasive carcinoma. However, this was not statistically significant. Using UCSC Genome Browser and PhastCons, we determined that *DEEBC* is highly conserved in mammalian genome. Further analysis did not reveal any *DEEBC* mutations in TCGA-breast cancer dataset (BRCA). Enrichment of H3K27AC-modified histones have been observed within this gene indicating the presence of active regulatory elements. Analysis of the TCGA samples did not show correlation of RNA levels with methylation status. In addition, the genes within 1 kilobase range on both sides of *DEEBC* gene locus showed no statistically significant expression changes in the ER+ breast and nodal met pairs.

Conclusions: The loss of *DEEBC* expression is an early event in development of ER-positive breast cancer. The loss is not due to mutations or methylation. It seems to function in a "trans" manner regulating function of genes that are not located in the neighbourhood. Further studies are necessary to understand the mechanisms of downregulation of DEEBC in breast cancer

164 Histopathologic Subgroups of AR+ TNBC Predict Pathologic Complete Response to Neoadjuvant Chemotherapy in Localized Breast Cancer

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Background: Approximately 10-20% of TNBCs express androgen receptor (AR) by immunohistochemistry (IHC). Expression profiling independently identifies these tumors as the luminal androgen receptor (LAR) subtype, characterized by luminal gene expression and driven by AR. Overall 30-40% of patients with TNBC achieve pathological complete response (pCR) after neoadjuvant chemotherapy (NAC); however, patients with LAR subtype BC have a poorer response with only 10% achieving pCR. To date histologic features permitting subset analysis of AR+ TNBC have not been described.

Design: Pre- and post-treatment tumor tissues from stage II/III patients with AR+ TNBC vs AR- TNBC accrued to a randomized phase II NAC trial of cisplatin and paclitaxel +/- everolimus were studied for histologic features (grade, TILs, AR and Ki67 expression) correlating with complete pathologic response (pCR). AR+ tumors were defined by $\geq$ 10% nuclear expression. TILs were scored by the method of Salgado et. al (Absent, Low < 10%, moderate 10-49%, high > 50%). The exact binomial test was used to assess statistical significance.

Results: Among 145 Stage II/III TNBC patients accrued between 2009 and 2013, 115 patients (2 with bilateral tumors) completed 12 weeks of therapy. 18% (20/117) of tumors were AR+. Histologically, they could be subdivided into 4 distinct subtypes: 1) Classic AR: High median AR+ (90%), large gland-forming infiltrative islands with abundant eosinophilic cytoplasm and prominent nucleoli or smaller eosinophilic glands in sclerotic stroma, moderate-low TILs [n=13]; 2) Low-AR: median AR+ (35%), solid, high grade, pushing borders, high TILs [n =5]; 3) Histiocytoid:100% AR+, low grade, absent TIL [n=1]; 4) Lobular:90% AR+, absent TIL [n=1]. AR+ tumors differed in response to NAC with 20% (4/20) AR+ vs. 42% (41 /97) AR- cases achieved pCR (p=0.0137). Among Classic-AR vs. Low-AR, 13% vs. 40% achieved pCR, and correlated with higher mean ki67 expression (p=0.0002) and trended with the presence of moderate-marked TILs (p =0.0692).

Conclusions: AR+ TNBC have lower rates of pCR than AR- TNBC and can be subdivided histologically into unique subgroups in which incorporation of Ki67 and TILs are highly predictive of pCR.

165 Analysis of *CDH1* in Invasive Lobular Carcinoma (ILC): Comparison of Morphology, Immunohistochemistry (IHC) and Mutation Profile Detected by Hybrid Capture-Based Next Generation Sequencing (NGS)

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Background: ILC comprises ~10% of breast cancers (BC), with classic (CL) and pleomorphic (PL) variants displaying distinct morphology. Alteration of the *CDH1* gene (16q22.1) with subsequent loss of the E-cadherin protein results in negative membranous staining by IHC, most common in ILC. This study compared the morphology, IHC and mutation profile of ILC with *CDH1* alteration.

Design: MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) utilizes hybridization capture and NGS to detect somatic alterations in exons and selected introns of common cancer related genes (currently 468). Invasive BC (>10% tumor) tested by MSK-IMPACT from 1/2014-6/2016 that yielded a *CDH1* alteration and/or had a diagnosis of ILC or invasive mammary carcinoma (IMC) with mixed ductal/lobular features were identified. Two pathologists reviewed available H&E/E-cadherin IHC slides and noted morphologic features characteristic of ILC.

Results: Out of 1361 BC analyzed, 241 BC met the above criteria and *CDH1* was altered in 80% (193/241). Mean overall coverage was good (*CDH1*-altered 680X, *CDH1*-unaltered 678X) and 38% (18/48) of *CDH1*-unaltered cases showed low tumor content. Upon review of morphology/IHC, the *CDH1*-altered cases include 77% (148/193) ILC (72% CL, 28% PL), 15% (29/193) IMC, and 8% (16/193) invasive ductal carcinoma (IDC). The *CDH1*-unaltered cases include 52% (25/48) ILC (64% CL, 36% PL), and 48% (23/48) IMC. Of cases with E-cadherin IHC (41%, 100/241), 93% (76/82) were IHC negative/*CDH1*-altered (68 ILC, 8 IMC), 7% (6/82) IHC positive/*CDH1*-altered (2 ILC, 3 IDC, 1 IMC), 61% (11/18) IHC negative/*CDH1*-unaltered (9 ILC, 2 IMC), 22% (4/18) IHC positive/*CDH1*-unaltered (4 IMC), and 17% (3/18) heterogeneous IHC/*CDH1*-unaltered (3 IMC). MSK-IMPACT also detected concurrent alterations in 292 and 148 genes for *CDH1*-altered and unaltered groups, respectively. Most common mutations in *CDH1*-altered group include *PIK3CA*, *TP53*, *TBX3* and *ERBB2* and in *CDH1*-unaltered group include *PIK3CA*, *TP53*, *MLL3*, *GATA3* and *MAP3K1*.

Conclusions: BC with morphologic features of ILC and negative E-cadherin IHC were most frequently *CDH1*-altered, showing good concordance among these 3 classification methods. Rare cases of IDC based upon morphology/IHC were *CDH1*-altered. Low tumor content, mixed tumors and mutations not covered by MSK-IMPACT (deep intronic, promoter methylation) may lead to a false *CDH1*-unaltered status, and morphology/IHC and concurrent genetic alterations could be most helpful in classifying these BC.

166 Reflex Alternate Reference Probe D17S122 in Double (IHC and FISH) *Equivocal* Invasive Breast Carcinomas May Aid in Establishing True HER2 Status

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Background: HER2 is an established prognostic factor and vital therapeutic target in the management of breast carcinomas. In 2013, the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines for HER2 evaluation were updated. Reflex testing using an alternate method is recommended on cases with equivocal results via immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH). However, therapeutic dilemmas arise when both tests are equivocal. The standard chromosome 17 centromere reference probe (CEP17) is in close proximity to the *HER2* locus and may be co-amplified leading to equivocal results. Alternate chromosome 17 reference probes may aid in establishing the true HER2 status. However, the current guidelines do not have a specific recommendation for use of these probes.

Design: Twelve patients with double equivocal invasive breast carcinomas (or discordant results) diagnosed in 2015-2016 were reviewed. Double equivocal results were defined as an initial HER2 IHC score 2+ as per current guidelines, and subsequent FISH with *HER2*/CEP17 ratio <2.0 and average 4 to <6 *HER2* signals/cell. Reflex FISH testing was performed with alternate reference probe D17S122 and classified accordingly.

Results: 10 of 12 cases (83%) had a definitive HER2 status with D17S122. 7 cases were ultimately classified as positive (58%) and 3 as negative (25%). 2 remained equivocal (17%). The HER2 status of our 2 cases with initially discordant results was also resolved by alternate probe testing. The first was double equivocal on biopsy and with subsequent IHC negative/FISH equivocal results on excision that was ultimately classified as HER2 positive with the alternate probe. The second was an IHC negative/FISH equivocal case that remained equivocal with reflex alternate probe testing.

Conclusions: 1. The use of alternate reference probe D17S122 yields definite results in over 80% of cases.

2. More than half of cases are ultimately classified as positive on reflex testing.

3. Reflex testing with alternate reference probes may establish true HER2 status and direct proper therapeutic management.

For optimal patient care, pathologists should utilize necessary alternate testing to resolve HER2 double equivocal cases. Additional studies are needed to further evaluate D17S122 and other available reference probes. Finally, the ASCO/CAP guidelines may need to be updated to reflect specific recommendations for the use of these probes in double equivocal cases.

167 Epithelial Proliferations in Juvenille Fibroadenomas

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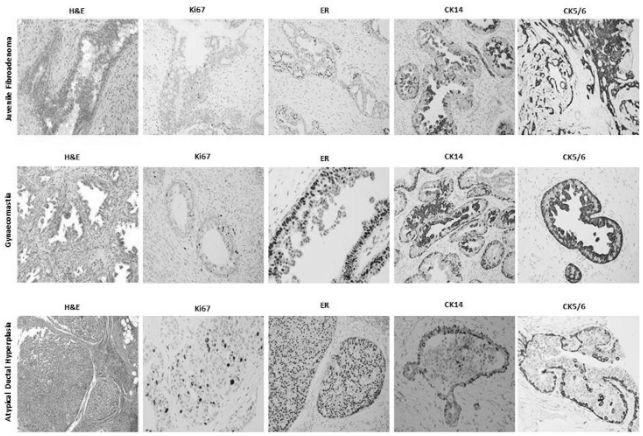
Background: 75% of breast tumours in children and adolescents are fibroadenomas of which 5-10% are juvenile/ giant fibroadenomas.

Many of these show varying degrees of epithelial proliferation which can be overcalled as atypical or malignant.

Aims: To study staining characteristics of epithelial proliferations within juvenile fibroadenomas using a panel of IHC (Immunohistochemistry) stains comprising ER, CK5/6, CK14 and Ki67.

To compare them on IHC to established cases of ADH (Atypical Ductal Hyperplasia)/ LGDCIS (Low grade DCIS) and gynaecomastia.

Design: We retrieved 50 cases each of Juvenile fibroadenomas, gynaecomastia and ADH/ LGDCIS from the pathology data bases of KK Hospital and SGH over a period of 15 years (2000- 2015). All slides were screened and Tissue micorarrays (TMAs) were constructed for each categorybased on the selected areas. These TMA cores were stained for ER, CK5/6, CK14 and Ki67.



Microphotographs exhibiting IHC staining characteristics of JFA, GM and ADH/ LG DCIS

Results: Statistical analysis was done using Kruskal-Wallis and Fisher's exact test.

Ki67 level was highest in ADH, followed by fibroadenoma and the lowest in gynaecomastia and all the differences were statistically significant.

ER, CK 14 & CK5/6 positivity was similar in Juvenille fibroadenoma's and gynaecomastia.

Factor	Median (IQR)/Frequency (Proportion)			P-value			
	ADH	FIBROADENOMA	GYNAECOMASTIA	Overall	FIBROADENOMA vs. GYNAECOMASTIA	ADH vs. GYNAECOMASTIA	ADH vs. FIBROADENOMA
KI67	7 (2.3, 12)	3 (1, 8)	1 (1, 2)	<0.0001	<0.0001	<0.0001	0.0124
ER	7 (6, 8)	3 (3, 4.8)	4 (3, 4)	<0.0001	0.2598	<0.0001	<0.0001
CK14				<0.0001	0.1259	<0.0001	<0.0001
Negative	40 (80%)	19 (38%)	11 (22%)				
Positive	10 (20%)	31 (62%)	39 (78%)				
CK5/6				<0.0001	0.6173	<0.0001	<0.0001
Negative	36 (72%)	3 (6%)	1(2%)				
Positive	14 (28%)	47 (94%)	48 (98%)				

Comparative analyses of JFA, GYN and ADH/LGDCIS.

Conclusions: We conclude that the epithelial proliferation in gynaecomastia and Juvenile fibroadenoma is similar and differs from ADH/LGDCIS. This can be confirmed by using a panel comprising CK14, CK5/6 and ER.

CK5/6 was superior to CK14 in confirming the benign nature of the proliferation in Juvenille fibroadenomas.

168 The Role of Integrin αvβ6 and MMP9 in Aggressive Clinical Behavior in Invasive Micropapillary Breast Carcinoma (IMPC)

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Background: IMPC of breast is an aggressive subtype with unique morphology of reversed cell orientation. αvβ6, an epithelial-specific integrin is a receptor for extracellular matrix, and may relate to EMT-like event. It is reported to promote tumor activity by activating TGF-β and matrix metalloproteinase 9 (MMP9), which makes the tumor microenvironment permissive to invasion. Our study evaluates and compares the role of αvβ6 and MMP9 in tumor cells and tumor microenvironment of the IMPC subtype with tubular carcinoma (TC) group, which has an indolent clinical course.

Design: Fifty-six cases (42 IMPC and 14 TC) were retrieved. IHC for EMA and D2-40 were performed to confirm IMPC by highlighting reversal of cell polarity and rule out lymphovascular invasion respectively. αvβ6 and MMP9 IHC staining were performed, and tumor-associated fibrosis and lymphocytic infiltrate evaluated and correlated with ER, PR, HER2 and lymph node status. Membranous staining for αvβ6 and both membranous and cytoplasmic staining for MMP9 were considered positive.

Results: Integrin αvβ6 expression was higher in IMPC (26.2%) versus TC (21.5%). Expression of αvβ6 in tumor cells was associated with lymph node metastasis (p=0.001) and tumor associated fibrosis (p=0.007). There was no stromal αvβ6 expressed. MMP9 was expressed in 23.8% of IMPC tumor cells, 19% of IMPC stromal cells, 14.3% of TC stromal cells and negative in TC tumor cells. The higher expression of MMP9 in IMPC tumor cells was statistically significant (p=0.05). MMP9 staining was inversely related to PR expression (p=0.056). ER and HER2 didn't show any relationship with the two markers. MMP9 expression was higher in tumours with increased intratumoral lymphocytes but was not statistitically significant.

	Integrin αvβ6		MMP9			
	Tumor cells		Stroma		Tumor cells	
	positive	negative	positive	negative	positive	negative
IMPC	11	31	8	34	10	32
TC	3	11	2	12	0	14
	P=1.000		p=0.687		p=0.05	

		integrin αvβ6		
		positive	negative	
LN Metastasis	positive	12	11	p=0.001
	negative	2	25	
Increased tumor associated fibrosis	present	3	26	p=0.007
	absent	13	16	

Conclusions: Expression of Integrin αvβ6 and MMP 9 is higher in IMPC. αvβ6 is significantly associated with lymph node metastasis and tumor fibrosis, which may play an important role in IMPC aggressive clinical behavior.

169 Estrogen Receptor Positive/Progesterone Receptor Negative/ (ER+/PR-) Breast Cancer (BC) Has a Poor Response to Endocrine Therapy (ET)

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Background: While about 70% of BCs are ER+, a small subset of these tumors have an ER+/PR- phenotype despite the fact that PR is an ER-inducible gene product. It has been previously demonstrated that the ER+/PR- BCs were generally associated with a worse clinical outcome when compared to the ER+/PR+ tumors, indicating that they are clinically and probably genetically different entities. In this study, we sought to further characterize the clinicopathologic features of ER+/PR- BCs and the prognostic outcomes in response to systemic therapy.

Design: The authors' institutional database was searched to identify ER+/PR- BCs from 1998 to 2013, using 1% as the cutoff value to define ER/PR positivity. The clinicopathologic parameters of the primary BCs were recorded, along with therapeutic modalities and outcomes. Analysis of distant relapse-free survival (RFS) was performed by using the Kaplan-Meier method and the log-rank test. Those with metastasis at diagnosis, synchronous BCs with a non-ER+/PR- component, those with an ER+/PR+ BC in the metastatic recurrence, and recurrent ER+/PR- tumors from ER+/PR+ BCs were excluded.

Results: A total of 589 ER+/PR- BCs met the inclusion criteria in the study period. Most traditional prognostic factors, including age, histologic grade, tumor size and nodal status, remained significant when applied to this subset of BCs. The H-score of ER expression, ranging from 1-300, was significantly higher in those without distant relapse (mean score 193 vs. 145; p=0.02). An H-score of <150 was associated with a significantly worse RFS in the entire cohort [HR 3.11; p=0.002] and in the subset of patients who received ET only [HR 3.78; p=0.02], whereas the addition of cytotoxic chemotherapy minimized the difference in RFS [HR 2.05; p=0.1]. This is in contrast to our previous findings in ER-/PR+ tumors, in which a PR H-score of 10 dichotomized prognostic outcomes [Hum Pathol 2015].

Conclusions: Our findings differ from a previous report that suggested that lack of PR expression is associated with low levels of ER [Breast Cancer Res Treat 2009]. This, along with the fact that estrogen-induced transcription of the PR gene does not always parallel ER occupancy, suggests that altered ER signaling in ER+/PR- BCs could contribute to ET resistance. Thus, these tumors require much higher ER levels to achieve a response to ET. While the current treatment for all ER+ tumors is similar, the observations thus far indicate that individualized systemic therapy should strongly be considered in early stages ER+ BCs given the additional compounding factors.

170 Utilizing Digital Specimen Tomography Improves Lesion Identification, Tumor Assessment, and Efficacy of Grossing of Breast Excision Specimens

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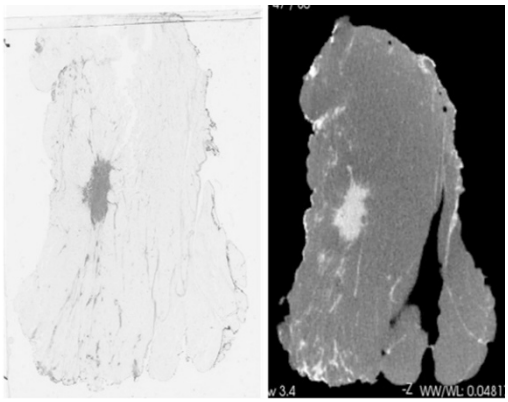
Background: Pathological evaluation of breast specimens relies critically on gross and radiographic findings. When a distinct lesion, biopsy site or clip is not grossly identified, misidentification of tumor or oversampling of tissue may result, often leading to inaccurate pathologic results and increased turn-around time. Digital Specimen Tomography (DST) is a new tool that is sufficiently compact and portable for potentially routine use in the gross room, particularly for breast specimens. It yields a full 3D image of tissue with up to 50-micron isotropic resolution in under 1.5 min. Our preliminary study evaluates DST's utility for improved accuracy of lesion identification and sampling, tumor extent and margin evaluation.

Design: We performed DST on 90 fresh, oriented breast lumpectomies with in-situ or invasive carcinomas. Then, specimens were measured, inked and serially sectioned. Grossly identified lesions were sampled using conventional grossing techniques without

DST influence. DST images were then reviewed; if DST gave additional information about lesions or a larger tumor extent than identified grossly, these areas were re-evaluated and submitted for histologic assessment.

Results: DST aided in identifying tumor, clip, previous biopsy site and/or calcifications in cases that were difficult to identify on gross inspection alone, which was particularly the case for in-situ lesions not palpated or visualized grossly. Suspicious lesions that were both identified grossly and via DST were indeed tumor. Compared to traditional grossing, significantly fewer number of tissue blocks were required for histologic examination. Finally, none of the specimens needed additional sections to be submitted.



Conclusions: DST is an effective, innovative adjunct to conventional grossing of breast excisions as it increases accuracy of sampling lesional tissue and resection margins and detects pathologic findings not identified grossly. Overall, by reducing both the rate of oversampling uninvolved tissue and the need for additional sampling, DST improves turn-around time and accuracy of pathologic parameters for breast specimens.

171 Occult Malignancy in Risk Reduction Bilateral Mastectomies from Women with BRCA1 or BRCA2 Germline Mutation: Correlation with Imaging Abnormalities and Implications for Specimen Sampling Strategies

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Background: The lifetime occurrence of breast cancer in women with a BRCA1 or BRCA2 germline mutation is significantly lowered by risk reducing bilateral mastectomy (RRBM), however early stage occult cancer may already be present in a minority of women at the time of RRBM. The incidence of occult cancer in RRBM varies widely in the limited literature. We hypothesize that the reported incidence may depend on patient age at surgery, radiologic imaging abnormalities, and specimen sampling strategy. Whereas an evidence-based protocol for specimen management of risk reducing salpingo-oophorectomy (RRSO) (so-called SEE-FIM protocol) has been established, a similar standardized protocol for managing RRBM remains to be defined.

Design: Clinical, radiologic, and pathologic findings were retrospectively evaluated in RRBM from 72 women with a BRCA1 or BRCA2 germline mutation who did not have any pre-operative pathologic diagnosis of atypia or malignancy of the breast. For patients who underwent imaging and biopsy, the correlation between BI-RADS score, biopsy findings, and RRBM diagnoses was evaluated. The specimen sampling approach for RRBM was 2 cassettes per quadrant plus sampling of any gross abnormality.

Results: Invasive ductal carcinoma with DCIS was present in 1/72 RRBM. The patient was 40 yrs (BRCA2), BI-RADS score 1 and had a benign biopsy. The tumor was 0.5 cm, grade 2, ER+PR+HER2-, pT1N0. Pure Paget disease was found in 1 patient, who was 37 yrs (BRCA2) with an ill-defined nodule on MRI. In the remaining 70 patients (average age 41 yrs), 54 had imaging: 38/54 had BI-RADS score 3 or less and 8/38 underwent biopsy; 16/54 (30%) had BI-RADS score 4 and 11/16 underwent biopsy. None were BI-RADS score 5. None of the biopsies contained atypia or malignancy, however, 7/70 RRBM contained ADH/FEA and 4/70 RRBM contained LCIS/ALH. Gross abnormalities were visible in 5/70 RRBM. The average number of cassettes per breast specimen was 12. RRSO was performed in 48 of 72 patients. 4/48 RRSO contained tubal/ovarian high grade serous carcinoma.

Conclusions: Occult cancer and atypia are rare in RRBM specimens, but neither radiologic imaging nor gross pathology correlated with its detection. Benign biopsy does not exclude occult cancer and specimen sampling strategies should still focus on grossly normal tissue. An evidence-based standardized protocol for management of RRBM specimens is needed; the optimal number of cassettes remains to be established.

172 Phenotypic Alterations in Breast Cancer Associated with Neoadjuvant Chemotherapy: A Comparison with Baseline Rates of Change

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Background: Several studies have documented phenotypic alterations in breast cancer associated with neoadjuvant chemotherapy [NACT]. Accordingly, there is an emerging consensus that the tumors in resection specimen be re-tested for estrogen receptor [ER], progesterone receptor [PR] and HER2/neu in this setting, irrespective of the testing status or results in the preceding biopsy. Prior studies are limited by the fact that they did not account for the baseline rate of expected phenotypic change between biopsies and resections in the absence of NACT. The primary goal of this study is to determine whether the rate of phenotypic alterations associated with NACT is significantly different than would be expected in a control population of patients who did not receive NACT

Design: From a pathologic database, we documented the ER, PR and HER2/neu phenotypes of all invasive breast carcinomas diagnosed at a single institution during a 6.5 year period. We determined the frequency of a phenotypic change in patients that received testing in both the biopsy and resection. Changes were assigned at the *negative* [-] vs *positive* [+] or *equivocal* threshold using current guidelines. We then compared the rates of phenotypic changes in the subsets of patients that did and did not receive NACT, as well as the direction of change in both groups

Results: From 826 cases of breast cancer diagnosed during this period, repeat testing (i.e. testing on both biopsy and resection) was performed in 340 (41%). 65 (19%) of these 340 patients had received NACT. A comparison of the NACT and the non-NACT groups regarding rates of change showed the following respective results. ER (9.2% vs 2.5%, p=0.02;); PR (30.7% vs 8%, p=0.000006); Her2/neu-IHC (25% vs 22.3%, p=0.7%), Her2/neu-FISH (7% vs 3%, p=0.6). The direction of biopsy-to-resection phenotype change in the NACT group was [+] to [-] in 83.3% (ER), 75% (PR), 87.5% (Her2/neu-IHC), 100% (Her2/neu-FISH). Parallel frequencies for the non-NACT group were 71.4% (ER), 73% (PR), 90% (Her2/neu-IHC), 67% (Her2/neu-FISH)

Conclusions: The current study compared the rate of breast cancer phenotypic change associated with NACT to a baseline of non-NACT patients that were similarly tested in both the biopsy and the resection. Although apparent phenotypic changes were identified for all markers, *significant* change above baseline was demonstrable only for ER and PR, as only these showed a rate of change associated with NACT that significantly exceeded the non-NACT group. This suggests that hormone receptors may be more susceptible to NACT-associated phenotypical changes than HER2/neu.

173 Are There Any Pathologic Features Associated with a Phenotypic Change After Neoadjuvant Chemotherapy in Breast Cancers?

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Background: Neoadjuvant chemotherapy [NACT] has previously been noted to change the phenotypes of a subset of breast cancers. As such, many institutions routinely perform repeat testing for estrogen receptor [ER], progesterone receptor (PR), and Her2/neu on resection specimens for patients with residual tumor after NACT. The goal of this study is to determine whether there is a pathologic profile that is significantly associated with a phenotypic change after NACT. Such a profile may theoretically facilitate the delineation of the subset of patients with residual disease after NACT that is most likely to have undergone a phenotypic change

Design: The study set included consecutive patients with invasive breast carcinoma who underwent NACT and whose cancers were resected with residual disease during a 6.5-year period. Changes in tumoral phenotype between the biopsy and resection regarding ER, PR, and HER2/neu were documented, as well as a wide array of clinicopathologic features, including patient age, tumor size, histotype, histologic grade, stage, DCIS component, lymphovascular invasion, lymph node status, necrosis, margins status, all as determined in the resection specimen. We compared the frequency of clinicopathologic parameters between the "phenotypically stable"[PS] and the "phenotypically altered" [PA] cases, with alterations defined at the "*positive* or *equivocal*" versus "*negative*" threshold using current criteria

Results: Of the 65 patients that received NACT with residual disease in the resection, 49.2% showed a change in status for at least one marker as compared with the prior biopsy (ER 9.2%; PR 30.8%; Her2/neu-FISH: 7%; Her2/neu-IHC: 25%). for ER, there was no statistically significant difference between the PA and the PS cases regarding all of the aforementioned clinicopathologic variables. For PR, the PS cases significantly differed from the PA cases only in their higher frequency of a DCIS component (67% vs 35%, p=0.03). For HER2/neu (IHC), the PA cases were more likely to be of ductal histotype than the PS cases (87 vs 73%, p=0.03). For HER2/neu (FISH), the PA tumors were smaller (means 0.3 vs 3.4 cm, p=0.0003), and more likely to be grade 1 (100 vs 11.5%, p=0.026) than the PS cases

Conclusions: Breast cancers that show phenotypic alterations in HER2/neu status after NACT are more likely to be low grade and of smaller size than those that are phenotypically stable. This may support the notion that small residual tumors after NACT commonly represent a biologically different clone than the original tumor, which highlights the need for repeat testing after NACT

174 ER, PR, and HER2/neu Testing in Breast Cancer: Quantifying the Value of Repeated Centralized Testing in Excision Specimens

Nosaibah Hariri, Andres A Roma, Farnaz Hasteh, Vighnesh Walavalkar, Oluwole Fadare. UCSD, San Diego, CA.

Background: Our practice setting is an academic tertiary care breast center, where most newly diagnosed cases of breast cancer were initially referred from an outside facility, and where accordingly, the diagnostic biopsies as well as estrogen/progesterone receptors and Her2/neu [ER/PR/HER2] status assessments were performed at these outside facilities. Like many such tertiary care centers, it is our institutional policy to repeat the tests for ER/PR/HER2 in resection specimens whenever these tests were performed on the preceding biopsy at an outside facility. This practice is ostensibly to maximize accuracy and to homogenize testing variables across our patient population. However, the evidentiary basis for this relatively widespread practice is limited. The goal of this study is to quantify the value of repeated testing, by assessing the frequency with which centralized results differ in a clinically significant fashion from the outside results

Design: We documented results from ER/PR/HER2 testing in consecutive invasive breast cancers over a 2.5 year period and analyzed the subset for which repeated testing for ER/PR/HER2 was performed in the resection solely due to the aforementioned institutional policy (i.e. because ER/PR/HER2 testing on the preceding biopsy was performed at an outside institution). The rate of biopsy-to-resection change in phenotype for each marker were calculated. "Change" was defined at the *negative* versus *positive* or *equivocal* threshold using current scoring guidelines.

Results: From 541 consecutive breast cancers, repeat testing was performed in 190. 37 had received neoadjuvant chemotherapy and were excluded. The rates and directions of biopsy-to-resection change for the remaining 153 were as follows: ER (1.3% [2/153], 100% from [+] to [-]); PR (6% [9/153], 78% from [+] to [-]); HER2/neu-IHC (22.6% [31/137]: 84% from score 2+ to 0/1+; 10% from 0/1+ to 2+; 6% from 3+ to 0); HER2/neu-FISH (2/61, 3.3%, 50% [+] to [-]; the single case changed to [+] required an alternate FISH probe to demonstrate amplification; the other was associated with multifocal cancer). There were no ER[-] *and* PR[-] biopsy cases that became ER and/or PR[+] in the resection. By coordinate analysis for the hormone receptors (i.e ER *and/or* PR[+] being indicative of "hormone receptor" positivity), none of the changes were clinically significant

Conclusions: HER2/neu status, as assessed by IHC, changed at a substantial rate. However, the negligible rates of a clinically significant change in ER or PR phenotypes call into question the value of routine repeat testing for ER and PR in this setting

175 Presence of Intra-Ductal Papillomas in Benign Breast Tissue and the Risk of Subsequent Breast Cancer in African American Women

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Background: Benign breast disease (BBD) is an established risk factor for developing breast cancer, and certain pathologic features are more strongly associated with breast cancer risk. Most of the studies evaluating BBD and breast cancer risk have been done in primarily white populations. Our study sought to describe this risk in African American (AA) cohort of women with BBD.

Design: Biopsies from AA women diagnosed with BBD from 1997 to 2009 were examined for 14 benign features, including the presence of intraductal papilloma (IDP) and followed for subsequent breast cancer. Women with IDP were matched to women without IDP (1:2 ratio) based on age at biopsy, year of biopsy, and biopsy type. The association between IDPs and the other pathologic features were compared using chi-square tests, and the risk of developing breast cancer was estimated using logistic regression and summarized with odds ratios (OR) and 95% confidence intervals (95% CI).

Results: A total of 3,360 AA women with BBD have been identified and their benign biopsies reviewed. 190 women subsequently developed breast cancer with a mean follow-up time of 11.5 years. Out of the total, our cohort included 1,368 biopsies; 456 with IDP (383 single and 73 multiple IDPs) and 912 without IDP. IDP was significantly associated with most of the other benign features evaluated (apocrine metaplasia, ductal hyperplasia, cysts, duct ectasia, fibroadenoma, radial scars, sclerosing adenosis, columnar alterations, and proliferation with and without atypia, all $p<0.001$) but not lobular hyperplasia, calcifications, or fibrosis. Of cases with IDP, 8% developed carcinoma while 6% of those without. 16% of cases with multiple IDP developed carcinoma versus 7% of those with single IDP. Multiple intraductal papillomas were associated with increased breast cancer risk (OR: 3.19, 95% CI 1.62 – 6.28), while single intraductal papillomas were not (OR: 1.18, 95% CI 0.73-1.92). When further adjusted for hyperplasia with atypia, the benign feature with the most established and strongest association with breast cancer risk, the risk associated with multiple IDPs was attenuated but still remained increased (OR: 2.19, 95% CI 1.08 – 4.47).

Conclusions: Among AA women, it appears that the presence of multiple intraductal papillomas is associated with an increased risk for the subsequent development of breast carcinoma. Understanding the etiology of intraductal papilloma development, and other associated BBD features, may provide insight into tumorigenesis.

176 Breast Cancer Histologic Grade and Histologic Subtype Impact Recurrence Score; Retrospective Review of 863 Oncotype Dx Results

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Background: As per NCCN guidelines, adjuvant chemotherapy may be considered for grade 2 & 3 tumors or any grade T1c tumor. Oncotype Dx test(ODx) may help in selecting patients with estrogen receptor positive tumors that may benefit from chemotherapy. Relationship of recurrence score(RS) with breast cancer histologic subtypes(HSu) and combined histologic grade (Gr) is unclear. Early results from TAILORx trial do suggest an association between Gr and RS in low to intermediate risk groups. We aimed to assess relationship of ODx results with Gr and HSu.

Design: Consecutive ODx results performed between 1/2007-7/2016 at two institutions were reviewed. Tumor HSu (WHO classification, 2012), Gr, RS and recurrence group (0-17=low, LR; 18-30= intermediate, IR; & >30=high, HR) were recorded. Frequencies were compared by Chi-Square test; p-value <0.05 considered significant.

Results: Total 863 ODx results were reviewed. There were 208(24.1%) Gr1, 540(62.6%) Gr2 & 115(13.3%) Gr3 tumors. There were 512(59.3%) LR, 294(34%) IR and 57(6.7%) HR tumors. No Gr1 high-risk tumor ($p< 0.001$) was identified.

Table 1. Histologic Grade Versus Recurrence Score

	Low (0-17), n (%)	Intermediate (18-30), n (%)	High (31-100), n (%)
Grade 1	141 (67.8)	77 (32.2)	0*
Grade 2	343 (63.5)	173 (32.0)	24 (4.5)
Grade 3	30 (26.1)	52 (45.2)	33 (28.7)

*P<0.001

All favorable histology (tubular/cribriform & mucinous; n=17) tumors except one were in LR or IR group. A pure mucinous carcinoma with RS of 35 (HR) on re-review showed micropapillary features. Invasive ductal and invasive micropapillary carcinoma showed slightly higher number of tumor in HR group than other subtypes (7.7% & 9% versus 2.5% & 1.6%; $p<0.001$).

Table 2. Histologic Subtype and Grade Versus Recurrence Score

	Low (0-17), n (%)	Intermediate (18-30), n (%)	High (31-100), n (%)	Total
Histologic subtype and grade				
Invasive ductal carcinoma (n=633)				
Grade 1	113 (69.8)	49 (30.2)	0	162
Grade 2	239 (63.1)	119 (31.4)	21 (5.5)	379
Grade 3	23 (25)	41 (44.6)	28 (30.4)	92
Subtotal	375 (59.2)	209 (33.0)	49 (7.7)	633
Invasive lobular carcinoma (n=121)				
Grade 1	9 (50.0)	9 (50.0)	0	18
Grade 2	53 (56.4)	39 (41.5)	2 (2.1)	94
Grade 3	3 (33.3)	5 (55.6)	1 (11.1)	9
Subtotal	65 (53.7)	53 (43.8)	3 (2.5)	121
Invasive carcinoma with ductal and lobular features (n=63)				
Grade 1	10 (71.4)	4 (28.6)	0	14
Grade 2	32 (78.0)	9 (22.0)	0	41
Grade 3	3 (37.5)	4 (50.0)	1 (12.5)	8
Subtotal	45 (71.4)	17 (27.0)	1 (1.6)	63
Mucinous carcinoma (n=12)				
Grade 1	6 (75.0)	2 (25.0)	0	8
Grade 2	2 (50.0)	1 (25.0)	1 (25.0)	4
Subtotal	8 (75)	3 (18.7)	1 (6.3)	
Mixed invasive mucinous and ductal carcinoma (n=6)				
Grade 1	0	2 (100)	0	2
Grade 2	2 (50.0)	2 (50.0)	0	4
Subtotal	2 (33.3)	4 (66.7)	0	6
Tubular/cribriform carcinoma (n=6)				
Grade 1	2 (50.0)	2 (50.0)	0	4
Grade 2	2 (100)	0	0	2
Subtotal	4 (66.7)	2 (33.3)	0	6
Medullary carcinoma (n=1)				
Grade 3	0	0	1 (100)	1
Invasive carcinoma with micropapillary component (n=21)				
Grade 2	12 (75.0)	4 (25.0)	0	16
Grade 3	1 (20.0)	2 (40.0)	2 (40.0)	5
Subtotal	13 (61.9)	6 (28.6)	2 (9.5)	21
Total	512 (59.3)	294 (34.1)	57 (6.6)	863

Conclusions: Our findings show that OCDx result is impacted by grade and histologic subtype of breast tumor. A high RS in a grade 1 tumor or in a tumor with favorable histology will be unusual, which should warrant further investigation. Grade and histologic subtype should be considered while ordering OCDx test.

177 Ki-67 Score Is Not Predictive of Overall Survival, Disease-Free Survival or Metastasis in Estrogen Receptor Positive, Her2 Negative, Lymph Node Negative Breast Cancers

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Background: Ki-67 staining score reflects the proliferation of cancers. Its role in estrogen receptor positive (ER+)/HER2- and lymph node negative (LN-) breast cancer is not defined.

Design: Totally 604 ER+/HER2-/LN-breast cancer cases with an Oncotype DX (ODX) recurrence score diagnosed from 2006-2014 were retrieved from our institution. Ki-67 score (percentage of positive tumor cells) was correlated with race (African American vs Caucasian), patient age, ODX recurrent score, ER and progesterone receptor (PR) expression, Nottingham tumor grade (1/2 vs 3), lymphovascular invasion (LVI), chemotherapy, hormonal and radiation therapy, overall survival (OS), disease free survival (DFS) and metastasis.

Results: Univariate analysis showed that high Ki-67 score was significantly associated with African American race, high ODX score, Nottingham tumor grade 3, presence of LVI, receiving chemotherapy, and younger age (all $P<0.05$). Univariate survival analysis showed that Ki-67 score was not correlated with OS, DFS, or metastasis (all $P>0.05$).

Conclusions: Although high Ki-67 score is correlated with adverse clinicopathologic features (high ODX score, Nottingham tumor grade 2, LVI and younger age), it does not seem to predict OS, DFS or metastasis in ER+/HER2-/LN- breast cancer.

178 Repeat Biomarker Testing (ER/PgR/HER2) in Grade 3 Breast Carcinoma – Not Truly Necessary

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Background: Current HER2 guidelines from 2013 American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) indicate HER2 testing must be repeated on a breast excision if the tumor is grade 3 and the biopsy is HER2 negative. The CAP 2014 template for biomarker reporting recommends consideration of repeat testing for negative HER2/ER/PgR, particularly when results are discordant with histologic findings. Published evidence to support these guidelines, however, is lacking. Our study examines the need for repeat biomarker testing in the subset of grade 3 breast carcinomas by comparing ER/PgR/HER2 status of biopsy and subsequent excision.

Design: Retrospective review at our academic institution from 2015-16 identified 75 grade 3 breast carcinomas with HER2 results on both biopsy and excision as well as 68 cases with ER/PgR repeat testing. HER2 was determined by immunohistochemistry and/or by FISH. Of note, our oncologists require repeat biomarker testing when initial testing is at an outside facility. Clinical management of patients with discordant results was reviewed.

Results: Of the 75 HER2 repeat tests, 73 had concordant results (97.3% HER2 concordance rate). Two discordant cases were negative on biopsy and equivocal on excision (average HER2 copy numbers of 4.42 and 4.9 signals/cell). These two patients were clinically managed as HER2 negative. Of the 68 ER/PgR test cases, 66 ER and 60 PgR cases showed concordant biopsy and excision results (97.1% ER and 88.2% PgR concordance rates). Of the two ER discordant cases, one negative case retested positive on excision (10% weak) and a positive case (5%, weak) retested negative on excision. Of the 8 PgR discordant cases, 2 positive cases retested negative on excision (5% weak, 15% moderate) and 6 negative cases retested positive on excision (2% weak, 2% moderate, 3% weak, 3% moderate, 15% strong, 8% moderate). Repeat ER/PgR test findings in the discordant cases did not affect endocrine therapy.

Conclusions: Our study demonstrates high concordance rates for ER/PgR/HER2 biomarker testing when comparing biopsy and excision results for grade 3 breast carcinomas. Retesting of biomarkers did not impact patient management in any studied case. In light of added time and cost of repeat testing and the high concordance rates, our study does not support current practice guidelines for repeat biomarker testing as it applies to grade 3 breast carcinoma.

179 Oncotype DX Testing Does Not Benefit Patients with Low-Grade Breast Invasive Carcinoma

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Background: Oncotype DX, a real-time polymerase chain reaction assay of 21 genes, generates a recurrence score (RS) to predict the prognosis and chemotherapy benefits for patients with ER-positive, HER2-negative invasive breast cancer. Magee equations are derived by linear regression analysis using several pathologic variables and semiquantitative immunohistochemical results to calculate a RS that highly correlates with the Oncotype DX RS and provides similar information to that from Oncotype DX. Several specific histologic types of breast carcinoma including invasive tubular carcinoma (ITC), mucinous carcinoma (IMC) and classical lobular carcinoma (CILC) have been considered as low-grade and to be associated with a favorable outcome. We aimed to examine Oncotype DX RSs and compare them with Magee equation RSs in these tumors.

Design: Oncotype DX RSs and pathological characteristics were collected and Magee equation RSs were calculated for 105 CILCs, 41 ITCs and 17 IMCs.

Results: Except two CILCs, all cases had Oncotype DX RS <30 (98.8%, 161/163), but all cases including those two CILCs had Magee equation RS <30. Overall, 105 (64.4%) cases had Oncotype DX RS <18, 56 (34.4%) cases had Oncotype DX RS between 18 and 30 and 2 (1.2%) cases had Oncotype DX RS > 30. 124 (76.1%) cases had Magee RS <18, 39 (23.9%) cases had Magee RS between 18 and 30 and no case had Magee RS > 30. The overall agreement between Oncotype DX RS and Magee RS was 68.7%. The two CILCs with Oncotype DX RS >30 had Oncotype DX RS of 32 and 36, but had Magee equation RS of 20.3 and 20.0. Both patients had been followed up for over 5 years, and no recurrence or metastasis occurred.

Table 1. Oncotype DX RS and Magee RS in all cases.

	Oncotype DX RS			Magee RS			Total
	<18	18-30	>30	<18	18-30	>30	
CILC	64	39	2	73	32	0	105
	61%	37%	2%	70%	30%	0%	100%
ITC	30	11	0	38	3	0	41
	73%	27%	0%	93%	7%	0%	100%
IMC	11	6	0	13	4	0	17
	65%	35%	0%	76%	24%	0%	100%
Total	105	56	2	124	39	0	163

Table 2. Correlation between Oncotype DX RS and Magee RS in all cases.

		Oncotype DX RS			
		<18	18-30	>30	Total
Magee RS	<18	90	34	0	124
	18-30	15	22	2	39
	>30	0	0	0	0
Total		105	56	2	163

Conclusions: Our findings suggest that performing Oncotype DX RS on these special types of tumors is unlikely to be useful to guide clinical management.

180 Assessing the Checkpoint Immune System (PD-L1, CD8 and CD163) in HER2-Positive Invasive Breast Carcinoma and Its Association with Clinical and Pathological Features

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Background: Programmed death ligand 1 (PD-L1) is an immune regulatory molecule that limits antitumor immune activity. PD-L1 expression by tumor cells and immune cells has been reported in breast cancer, especially in triple negative breast cancer. In this study, we aimed to evaluate the checkpoint immune system in HER2-positive breast cancer and its association with clinical/pathological profiles.

Design: 216 HER2-positive invasive breast carcinomas without neoadjuvant chemotherapy were included in our current study and tissue microarrays (TMA) were constructed in duplicate from surgical specimens. Multi-color multiplex immunohistochemistry with co-localization of PD-L1 with other immune markers (CD8 and CD163) was performed on TMA sections and the following parameters were assessed; PD-L1-expressing tumor cells (PD-L1 TC), PD-L1-expressing peritumoral inflammatory cells (PD-L1 PTIC), PD-L1-expressing intratumoral inflammatory cells (PD-L1 ITIC), tumor infiltrating lymphocytes (TIL), peritumoral T cells (PTT), peritumoral macrophages (PTM) and tumor associated macrophages (TAM). PD-L1 staining was scored with a positive threshold of staining in ≥5% of tumor cells or immune cells.

Results: PD-L1 expressionin tumor cells or immune cells was observed in 13% of total cases. PD-L1 expression was strongly associated with peritumoral/intratumoral lymphocytes/macrophage infiltrates. Additionally, PD-L1 expression was associated with high Nottingham grade, high nuclear grade, high mitotic activity, ER negativity, but with lower tumor stage and less frequent lymph node metastases. More importantly, breast cancers with PD-L1 expression showed less frequency of local recurrence and distal metastases.

Conclusions: Our data suggest that PD-L1 expression is associated with a more aggressive biology, but may play favorable predictive and/or prognostic roles in HER2-positive invasive breast carcinoma.

		PD-L1 positive # (%) /average (range)	PD-L1 negative # (%) /average (range)	Total # (%) /average (range)	p value
Case #		29	187	216	
Age (years)		55.0 (28-88)	53.9 (27-86)	54.1 (27-88)	NS
Checkpoint immune system	PD-L1+ cases (≥5%)	29 (100%)	0 (0%)	29 (13%)	<.001
	PD-L1 TC	12 (41%)	1 (0.5%)	13 (6%)	<.001
	PD-L1 PTIC	29 (100%)	6 (3%)	35 (16%)	<.001
	PD-L1 ITIC	9 (31%)	1 (0.5%)	10 (5%)	<.001
	PTM	27 (93%)	62 (33%)	89 (41%)	<.001
	PTT	29 (100%)	42 (22%)	71 (33%)	<.001
	TIL	11 (38%)	14 (7%)	25 (12%)	<.001
	TAM	11 (38%)	39 (21%)	50 (23%)	<.001
Pathologic features	IDC	28 (97%)	172 (92%)	200 (93%)	NS
	Nottingham grade	2.86 (2-3)	2.53 (2-3)	2.58 (2-3)	0.004
	Tubule	3.00 (2-3)	2.84 (2-3)	2.87 (2-3)	NS
	Nuclear	3.00 (2-3)	2.70 (2-3)	2.76 (2-3)	0.022
	Mitotic	2.50 (1-3)	2.01 (1-3)	2.10 (1-3)	0.021
	ER-	18 (62%)	71 (38%)	89 (41%)	0.014
	PR-	19 (66%)	97 (52%)	116 (54%)	NS
Surgical features	Lumpectomy	16 (55%)	77 (41%)	93 (43%)	NS
	Mastectomy	13 (45%)	110 (59%)	123 (57%)	
	Tumor size (cm)	2.13 (0.7-5.0)	2.49 (0.3-13)	2.44 (0.3-13)	NS
	Margin positive	1 (3%)	11 (6%)	12 (6%)	NS
	LVI present	8 (28%)	79 (42%)	87 (40%)	NS
T staging	T1	16 (55%)	100 (53%)	116 (54%)	NS
	T2	13 (45%)	71 (38%)	84 (39%)	NS
	T3/T4	0 (0%)	16 (9%)	16 (7%)	0.046
Lymph nodes	Cases #	29	181	210	
	Sentinel LN only	14 (48%)	54 (30%)	68 (32%)	0.049
	Axillary LN	15 (52%)	127 (70%)	142 (68%)	
	LN positive	6 (21%)	91 (50%)	97 (44%)	0.005
Follow-up	Cases #	7	79	86	
	Local recurrence	0 (0%)	9 (11%)	9 (10%)	NS
	Distal metastasis	0 (0%)	20 (25%)	20 (23%)	0.064
	Local/distal	0 (0%)	29 (37%)	29 (34%)	0.049

Table 1. Clinical and pathological features in PD-L1-positive and PD-L1-negative HER2-positive invasive breast cancer. Abbreviations: PD-L1 TC: PD-L1-expressing tumor cells; PD-L1 PTIC: PD-L1-expressing peritumoral inflammatory cells; PD-L1 ITIC: PD-L1-expressing intratumoral inflammatory cells; PTM: peritumoral macrophages; PTT: peritumoral T cells; TIL: tumor infiltrating lymphocytes; TAM: tumor associated macrophages; IDC: invasive ductal carcinoma; LVI: lymphovascular invasion; LN: lymph node; NS: no statistical significance.

181 Decrease the Overuse of ER PR Immunohistochemistry Studies in Pure Low Grade Ductal Carcinoma In Situ (DCIS)

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Background: Pathology laboratories in the United States are experiencing significant financial challenges nowadays. However, an increasing demand for immunohistochemistry to aid pathologic diagnostics or clinical treatments exacerbates the situation. The possibility of decreasing overuse of immunohistochemistry (IHC) studies in certain situation will help solve the dilemma. Low grade ductal carcinoma in situ (DCIS) frequently show diffuse and strong expression of estrogen receptor (ER) and progesterone receptor (PR). ER has been wildly accepted as a useful biomarker for potential benefit of adjuvant hormonal therapy (tamoxifen) in hormone receptor-positive DCIS. ER expression is also a predictive marker of the effectiveness of tamoxifen in the treatment of DCIS. There is a controversy regarding whether routine PR testing is necessary.

Design: In this study, we explore 100 cases with low grade DCIS only (core needle biopsy or excision specimen) and analyze their expression profile of ER, PR by IHC. For both ER and PR a positive results is defined as >1% of cells showing nuclear staining.
Results: We find that 100% (100 of 100) cases are positive for ER. Eighty six cases are given a score based on the expression intensity on a scale of 1 to 3 (3 being strong expression), out those 86 cases, 95% (82 of 86) have a score of 3 (strong expression). The rest 5% have a score of 2 to 3. Similarly, 100% (100 of 100) are positive for PR, 74% (63 of 86) have score of 3 for PR.
Conclusions: We should consider ER and PR hormonal status to be positive (majority with strong expression) in low grade DCIS specimen, and stop ordering these stains!

182 Flat Epithelial Atypia on Core Biopsy Is Not Associated with Upstage at Excision

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Background: The management of isolated flat epithelial atypia (FEA) on breast core biopsy is controversial. Early studies demonstrated the risk of upstage to invasive carcinoma or ductal carcinoma in situ (DCIS) to be similar to that of atypical ductal hyperplasia (ADH), supporting management of FEA with excision. However more recent studies have shown this upstage rate to be much lower, suggesting that these lesions may not require excision. Most studies of FEA to date have been small. We present a retrospective review of 115 core biopsies with FEA and corresponding excision data.
Design: After Institutional Review Board approval, 252 breast core biopsies with a diagnosis of FEA from 1 January 2010 to 30 September 2015 were retrieved from the Anatomic Pathology information system (CoPath). Slides were available in 230 cases. Lack of excisional biopsy results in 22 cases resulted in 208 cases for analysis. Following review of cases to exclude co-existing ADH, ipsilateral DCIS or invasive carcinoma, and confirmation of diagnosis by two dedicated breast pathologists, 129 cases of FEA were confirmed. Of these 129 cases, biopsies done for calcifications on mammography were included (n=115); biopsies performed for other indications were excluded.
Results: 115 core biopsies with FEA meeting our inclusion criteria were identified. 19 of these (16.5%) also demonstrated atypical lobular hyperplasia (ALH) on core biopsy. In subsequent excisions, 0/115 (0%) showed invasive carcinoma or DCIS, 20/115 (17.4%) had ADH, 6/115 (5.2%) had lobular carcinoma in situ (LCIS), and 23/115 (20%) had ALH. When cases with ALH on core biopsy were excluded, 0/96 (0%) showed invasive carcinoma or DCIS, 18/96 (18.8%) had ADH, 3/96 had LCIS (3.1%), and 16/96 (16.7%) had ALH.
Conclusions: In our series of 115 cases, FEA on core biopsy was not associated with upstage to invasive carcinoma or DCIS at excision. The presence of ALH did not influence the upstage rate of FEA. Follow up imaging or anti-estrogen therapy may be a reasonable alternative to excision.

183 Calidoscope: A Three-Dimensional Tool for Quality Control in Breast Cancer Pathology

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Background: The human epidermal growth factor receptor 2 (*HER2*) gene is amplified and/or overexpressed in approximately 15% to 20% of primary breast cancers. Interlaboratory variability in HER2 testing is a challenge for targeted therapy in breast cancer patients. Assessment of positivity rates among laboratories, external quality control strategies, training courses for HER2 testing and the ConfirmaHER2 (that define reference values for positivity rates in Spain) are powerful tools to improve HER2 determination. These systems are also useful to identify laboratories that require further support.
Design: We have designed an algorithm called Calidoscope that integrates the HER2 global information obtained from ConfimaHER2, Spanish Quality Control Program and online Biomarkers Interpretation Module of Spanish Pathology Society to detect HER2 variation among pathology departments and pathologist interpretation.
Results: Calidoscope detects centers with technical problems, variability at determination level and centers "outsiders" from ConfirmaHER2 tool. In collaboration with the Companies we will use this information to solve first the technical problems. After that, we will focus on pathological interpretation of HER2, offering different options: training, sharing cases with experts and meetings.
Conclusions: Although regular participation in proficiency testing, update online courses to improve HER2 determination and recommendations for HER2 testing have significantly improved its implementation, errors are still present. We have designed an algorithm to improve this determination, acting at technical and interpretative level, with the implication of the companies involved and in close collaboration between centers and Quality Control Program.

184 Increased FoxP3-Positive Tregs Augur Better Survival and Colocalize with Both $CD8^+$ T-cells and $CD20^+$ B-cells within the Microenvironment of Triple Negative Breast Cancer

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Background: The roles of specific immune cell populations particularly the role of Forkhead Box Protein 3 (Foxp3) expressing regulatory T cells (Tregs) as well as B-cells in breast cancer remain unclear. We examined the abundance and localization of total T cells, B cells, and Tregs as well as mRNA levels within samples from triple negative breast cancers and asked whether these parameters were associated with pathological features of the cancer or clinical outcomes.
Design: Total of 164 samples of triple negative breast cancers diagnosed between 2003 and 2010 in Singapore were divided into "high" and "low" intra-tumoral or stromal groups, based on higher or lower- than-median densities of specific tumor-infiltrating lymphocyte populations ($CD3^+$ total T cells, $Foxp3^+CD3^+$ Tregs, or $CD20^+$ B cells) in the intra-tumoral space or stroma. A quantitative, digital gene expression NanoString assay was used to measure expression of a panel of 770 cancer-associated genes in samples from 11 high intra-tumoral Treg and 11 low intra-tumoral Treg triple negative breast cancers.
Results: Tumors with high densities of $Foxp3^+$ Tregs within their intra-tumoral, but not stromal, areas experienced significantly longer overall and disease-free survival. These "high intra-tumoral Treg" tumors were also characterized by relatively higher frequencies of $CD8^+$ T cells and $CD20^+$ B cells whcich colocaized with the Tregs. Ingenuity Pathway Analysis of the 31-gene signature seen in the high Treg group of triple negative breast cancers showed 9 genes associated with inflammatory response, immune cell trafficking and cell mediated immune response, in contrast to low Treg tumors.
Conclusions: Combination of high densities of intra-tumoral Tregs, $CD8^+$ T cells and $CD20^+$ B cells represents a favorable prognostic panel in triple negative breast cancers. These data indicate new avenues for further investigation on the interaction between tumor immune cell types. Also particular microenvironment of a triple negative breast cancer may attenuate the Tregs immunosuppressive functions; future studies in this area will be of significant interest.

185 Roles of PD-L1, PD-L2 and PD-1 in Triple Negative Breast Cancers: Perspective from an Asian Cohort

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Background: Antibodies inhibiting PD1/PDL1 co-inhibitory pathway molecules have shown promising results in triple negative breast cancers (TNBC) clinical trials. Hence, the knowledge of the expression and distribution of PD-L1, PD-L2 and PD-1 in the tumor microenvironment is critical for identifying a predictive as well as prognostic signature.
Design: 323 TNBCs diagnosed between 2003 and 2013 in Singapore General Hospital were stained with anti-PD-L1 antibody (E1L3N). The same cohort was subjected to quantitative, digital gene expression NanoString assay to measure expression of a panel of 499 cancer-associated genes. The cohort was divided into "PD-L1-positive" and "PD-L-negative" based on PD-L1 mRNA (*CD274*) expression. Clinicopathological parameters were correlated with protein and mRNA expression.
Results: At the protein level, the tumoral expression of PD-L1 was 17.6% in our TNBC cohort and these patients experienced significantly longer disease-free survival (DFS) ($p=0.03$) but not overall survival (OS) compared to patients bearing PD-L1-negative tumors. Multivariate survival analysis further confirmed the finding (HR 0.28, $p=0.002$). The protein and mRNA expression of PD-L1 was well correlated ($R=0.448$, $p=0.04$). At the transcription level, patients with *CD274* positivity showed non-statistically significant association with better OS ($p=0.06$) and DFS ($p=0.07$). However, statistically significant results were observed when *CD274* was combined with both PD-L2 (*PDCD1LG2*) and PD-1 (*PDCD1*) mRNA expression profile (OS and DFS, $p<0.001$). According to IPA canonical pathways analysis, these "PD-L1-positive" tumors were characterized by relatively higher expression levels of genes associated with crosstalk between dendritic cells and natural killer cells ($P<0.001$, 83.3 overlapped) as well as communication between innate and adaptive immune cells ($P<0.001$, 80.0 overlapped).
Conclusions: PD-L1 expression in tumor cells was associated with longer disease-specific overall survival in our TNBC data set in both univariate and multivariate analyses. A three-gene signature of *CD274*, *PDCD1LG2* and *PDCD1* mRNA expression demonstrated the potential as a better prognostic indicator of clinical outcome in TNBC.

186 Patterns of Recurrence in African-American Women with Luminal A and Luminal B Subtype Breast Cancer - A Single Institution Experience

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Background: Triple negative breast cancer (TNBC) recurrence has been extensively studied in African-American (AA) women because of the high frequency of recurrence. But not much is known about the recurrence of luminal A (LA) and luminal B (LB) subtypes in this population. We describe here the frequency of recurrence and time to recurrence for LA and LB subtype breast cancer in AA women.
Design: We describe data collected on 96 AA women with lymph node negative disease at diagnosis in whom histopathologic diagnosis, ER, PR, HER 2 and Ki67 results were reviewed and cases had been classified into either LA or LB subtypes. We included all cases where follow-up data existed for at least nine years.
Results: Of the 96 total cases, 89 cases were invasive ductal carcinomas, 5 were invasive lobular carcinomas and 2 were mixed with features of both. Based on molecular analysis, 14 cases were classified as LA subtype and 82 as LB subtype. During the follow up period, there were 3 recurrences in the LA group (21%, 95% CI: 1% - 42%) and 20 recurrences in the LB group (24%, 95% CI: 15-33%).
In patients with LA type, one patient experienced recurrence at 34 months and two patients experienced late recurrence after 5 years (148 months and 168 months). In patients with LB subtype, median time to recurrence was 95 months, with late recurrences occurring in 16 out of 20 patients (80%).
Conclusions: This is a single institution report of 96 cases of lymph node negative breast cancer in AA women. In the overall population, recurrence rates for LA and LB subtypes are reported to be 14% and 24% respectively (Metzger-Filho et al, 2013). Our experience suggests that unlike TNBC, AA women with LA and LB subtypes likely have similar recurrence rates as the overall population, though conclusions are limited by the small number of patients with LA subtype.

187 Deep Learning Automated Segmentation of Tumor Infiltrating Lymphocytes in Breast Cancer Specimens

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Background: Increased densities of tumor infiltrating lymphocytes (TIL) are associated with better patient outcome in solid tumors including breast cancer. We hypothesized that quantitative histomorphometry using digital image processing may improve standardization, intra- and inter-observer variability, and allow assessment of the spatial architecture of TILs. The aim of this pilot study was to develop adeep and machine learning algorithm to quantify and spatially analyze TILs from H&E stained slides of breast core needle biopsies.

Design: Twelve regions of interest (ROIs) were extracted from digitized H&E sections of breast cancer cases representing a spectrum of low, intermediate and high TILs and manually annotated for lymphocytes. Automated Deep Learning nuclei segmentation was compared with manual segmentation in 10 ROIs to separate lymphocytes and tumor/stromal cell nuclei.

Results: 43 features, including shape features such as perimeter to area ratios, textural features such as mean Haralick intensity, and color features such as red (eosin) to blue (hematoxylin) ratio were extracted for each nucleus and used to train several classifiers, including Random Forest, Neural Network, and Support vector models using 3-fold cross validation.The Random Forest model was the highest performing TILs classifier with an area under the receiver operating curve of 0.87 (figure1) for separating tumor/stromal nuclei and lymphocytes (figure2).

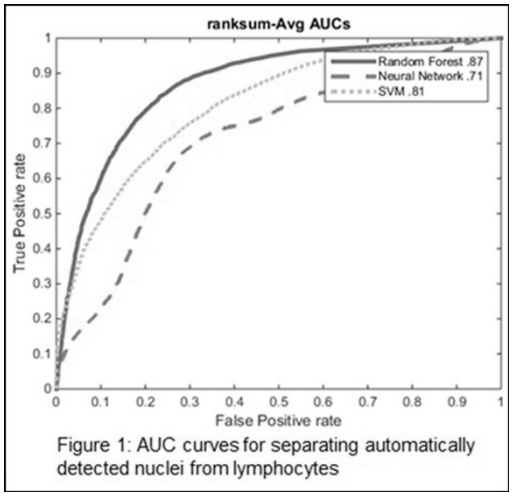

Figure 1: AUC curves for separating automatically detected nuclei from lymphocytes

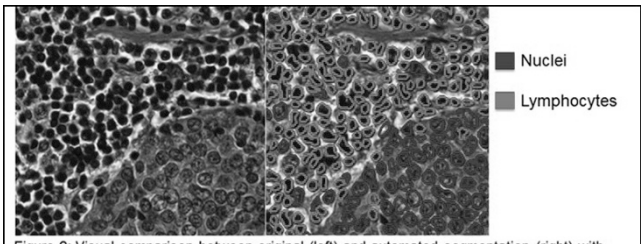

Figure 2: Visual comparison between original (left) and automated segmentation (right) with Automatically segmented nuclei (blue) and lymphocytes (red).

Conclusions: We developed a fully automated lymphocyte detection algorithm to aid in TIL assessment of breast cancer specimens. Further studies are needed to assess the predictive and prognostic capabilities of this classifier in larger breast cancer cohorts.

188 Gene Expression Analysis of Immune Response in Triple Negative Breast Carcinomas Among Different Ethnic Groups

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Background: Triple negative breast cancers (TNBC) are aggressive heterogeneous tumors which need to be refined to identify therapeutic subsets. Very limited data is available on predictive immune response markers in different ethnic groups. Our study aims to investigate the expression of immune cell infiltration markers and their prognostic significance in TNBCs among Caucasian (CA) and African American (AA) ethnic groups.

Design: Baylor, Scott & White database was searched, 47 AA and 37 CA TNBC cases with 5-15 years of follow up and treatment data were identified. Total mRNA was extracted (Qiagen, MD) and mRNA expressions of 785 breast cancer-related genes were quantitated and analyzed using nSolver Analysis Software (V2.5). TMA of corresponding tumor sections was subjected to CD68 IHC and quantitated using Applied Spectral imaging.

Results: Gene expression profile shows upregulation of STAT1, CXCL9, MX1 and IFITM1 in both groups [table1]. PD-L1, CXCL10, FOXP3, IRF1, MX1, IFIT1, IFITM1 and IFN-γ showed statistically significant difference in expression with p-values <o.1 (table 1) as well as correlated with presence of metastasis (p value: 0.0415) and recurrence (p value: 0.003) in AA subset.

Table 1: Selected immune cell infiltration gene panel

GENE	mRNA expression Mean (AA subset)	mRNA expression Mean (CA subset)	p-values
STAT-1	215.835	171.682	0.198
SOCS1	13.183	10.420	0.209
IRF1	31.089	20.565	0.042
CXCL9	183.954	166.436	0.416
CXCL10	45.482	28.348	0.074
CXCL11	55.783	48.381	0.349
IFIT1	38.466	29.030	0.094
IFITM1	278.226	191.245	0.009
MX1	308.479	182.336	0.004
CD68	72.070	60.842	0.665
CD163	67.020	56.452	0.644
PD-L1	8.452	6.113	0.070
PD-L2	6.342	6.036	0.488
PD1	9.033	7.185	0.196
IFN-γ	14.319	20.711	0.048
FOXP3	9.757	6.617	0.034

CD 68 IHC showed presence of increased Tumor Associated macrophages (TAMs) as a component of tumor infiltrating lymphocytes (p value:0.0017) and in the stroma (p value: 0.0005) of AA subset.

Conclusions: Our study identified significant differential expression of immune markers including PD-L1 and increased presence of TAMs in the AA subset which correlates with significantly unfavorable outcome. Our data suggests TAMs and other immune effectors could serve as useful biomarkers of outcome and therapeutic targets in the AA subset of TNBCs.

189 A Comparison Between the Clinicopathological Features of HER2 Positive versus HER2 Negative Invasive Lobular Carcinomas: A 7 Year Retrospective Analysis

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Background: Invasive lobular carcinomas (ILC) are typically HER2 negative. HER2 positive ILCs are rare and are reported to be associated with pleomorphic type. In this study, we retrospectively reviewed ILC cases over the past seven years and assessed their HER2 status. We reviewed their pathological features and clinical follow-up data.

Design: All ILC cases in our institution between 2010 and 2016 were retrieved from the pathology electronic record system. HER2 positivity was confirmed by FISH study using the 2013 ASCO/CAP guideline. Histopathologic data and lymph node status was collected as well as estrogen receptor (ER) and progesterone receptor (PR) status. Clinical information was also collected such as age and incidence of recurrence and survival.

Results: A total of 70 ILC cases were identified with 9 cases being HER2 positive (12.9%). The clinicopathologic data for 9 HER2 positive and 44 HER2 negative cases are shown in Table 1. All HER2 positive patients were treated with Herceptin clinically.

	HER2 positive ILC (n=9)	HER2 negative ILC (n=44)
Mean Age (years)	57 (range 50-75)	61 (range 36-81)
Nuclear Grade	1: 02: 83: 1	1: 42: 403: 0
Histologic Grade	I: 0II: 8III: 1	I: 4II: 40III: 0
Histologic subtype	Classic type: 8Mixed type: 1	Classic type: 38Mixed type: 6
Lymphovascular invasion	Present: 0; Absent: 9	Present: 5; Absent 39
Axillary Lymph Node	N0: 6N1a: 3N2a: 0N3a: 0	N0: 28N1a: 6N2a: 0N3a: 10
ER >90% 10-90% <10%	612	3851
PR >90% 10-90% <10%	522	20915
Follow-up (months)	Range: 5-67	Range: 12-67
Recurrence	Yes: 0No: 9	Yes: 0No: 44
Death	Yes: 0No: 9	Yes: 0No: 44

Conclusions: HER2 positive ILCs do not have specific morphologic features. They are intermediate grade and share similar ER/PR profile with HER2 negative ILCs. The short-term follow-up study demonstrates comparable clinical outcomes between these two entities.

190 PD-L1 in Breast Cancer: Comparative Analysis of Three Different Antibodies

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Background: Programmed cell death ligand 1 (PD-L1) encoded by the CD272 gene on chromosome 9, is expressed on a variety of normal cells including NK, macrophages, dendritic, B, epithelial and endothelial cells. The PD-1/PD-L1 axis protects the host from activated T-effector cells in cancer and other microbial infections. Targeting the PD-1/PD-L1 pathway may prevent inhibitory T-cell signaling and reactivate T cells to mediate tumor killing. The value of PD-L1 detection by immunohistochemistry (IHC) as a valuable marker is confounded by many unresolved issues such as different detecting antibodies, different staining protocols and platforms and different cutoff

points in addition to variable tissue preparations and variable tumors with different characteristics. The aim of this study is to compare the expression and performance of three commercially available PD-L1 antibodies in breast cancer.

Design: IHC analysis for PD-L1 in tumor using Ventana (SP263), Dako (IHC22C3) and Biocare antibodies (CAL10) was compared on 138 specimens including 43 primary tumors, 48 metastatic in regional LNs (43 paired to primary tumors) and 47 non-paired distant metastases. PD-L1 expression was correlated with several parameters including tumor size, histologic grade, ER, PR, Her2, Ki67, molecular type, TN status.

Results: The three antibodies performed equally well. Of the 138 comparisons between the three antibodies, only in four instances was there a discrepancy in terms of classification as PD-L1-negative (0% staining) vs PD-L1-positive (>1-100% staining). This high concordance is qualified by the fact that 86% of specimens were uniformly negative. 96% of the positive samples were TN tumors. The four differences between antibodies did not exhibit any pattern, with one in a distant metastasis from a TN primary, one from a regional LN from a Her2 primary, and two from a paired primary and regional LN of TN subtype. Moreover, there was no consistency as to which antibody was more likely to classify as positive vs negative. Given the high concordance, it is not surprising that all three antibodies demonstrated the same associations with all pathologic and clinical parameters studied.

Conclusions: We sought to identify a reliable method to assess PD-L1 expression in breast cancer. All three studied antibodies exhibited similar if not identical performance. In our view, pathologists have the option of utilizing less expensive reagents for the evaluation of PD-L1 in breast cancer.

191 Progesterone Receptor Expression Is More Prognostic Than Estrogen Receptor and Ki-67 Expression in Invasive Lobular Carcinoma Compared to Invasive Ductal Carcinoma: A Multi-Institutional Study

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Background: Invasive lobular carcinoma (ILC) differs from invasive ductal carcinoma (IDC) with respect to morphologic, clinical and molecular features. It has been previously reported that immunophenotypic features may not have the same prognostic value for ILC that they have for IDC. Interest in the relationship between progesterone receptor (PR) and Ki-67 expression in invasive breast cancer has been increasing. We examined the relationship between the Oncotype DX recurrence score (ODXRS), estrogen receptor (ER), PR and Ki-67 in ILC and IDC.

Design: 575 consecutive IDC's and 103 consecutive ILC's with available Ki-67, PR, ER, and ODXRS were identified from the pathology files at two separate institutions from 2008-2016. We examined relationships between the ODXRS, Ki-67 expression, and the modified H-score [Turner et al. Mod Pathol. 2015] for PR and ER.

Results: We found no significant difference (see Table) in the ODXRS (p=0.09) or the modified ER H-score (mERH, p=0.87) between ILC and IDC. We found a significant difference in the modified PR H-score (mPRH, p=0.009) and Ki-67 ($p < 0.001$) between ILC and IDC. ***ILC*** and *IDC* had a significantly stronger correlation ($p < 0.05$) between the mPRH and ODXRS (***R^2= 0.18 [ILC]***; *R^2= 0.24 [IDC]*) compared to the correlation between the mERH and ODXRS (***R^2= 0.03 [ILC]***; *R^2= 0.12 [IDC]*), with ILC having the stronger difference in correlation. We found a significantly stronger correlation ($p < 0.05$) between Ki-67 and the ODXRS in IDC (R^2= 0.33) compared to ILC (R^2= 0.01). Ki-67 was significantly more correlated ($p < 0.05$) with the *mPRH and mERH* in IDC (R^2= *0.06 and 0.09*, respectively) compared to ILC (R^2= *0.001 and 0.002*, respectively).

Prognostic variable	ILC (mean)	IDC (mean)
ODXRS	16.2	17.6
mERH	254.4	255.4
mPRH	144.4	176.2
Ki-67	9.6	15.3

Conclusions: PR expression may be more prognostic in predicting breast cancer recurrence (BCR) than ER and Ki-67 expression in ILC compared to IDC. While Ki-67 clearly has prognostic value in IDC, the prognostic value of Ki-67 in ILC is less clear. ODXRS estimates risk of BCR, and is heavily weighted on the expression of ER, PR, and tumor proliferation. Weaker correlations between Ki-67 with ER and PR in ILC compared to IDC suggest that the ODXRS may underestimate risk of BCR in patients with ILC. We continue to identify additional cases, with clinical outcomes, to further test this hypothesis.

192 Strong Expression of HSP27, HSP60 and BCL2 Associated with Luminal Subtypes of Breast Ductal Carcinomas in African American Women

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Background: Heat shock proteins HSP27 and HSP60 are antiapoptotic proteins encoded by mitochondrial genome, expressed during cell stress. BCL2 inhibits apoptosis. Our objective was to evaluate HSP27, HSP60 and BCL2 expression by immunohistochemistry in the four major subtypes of breast carcinoma (Luminal A, Luminal B, HER2, and Triple Negative) in a population of 202 African-American (AA) women with other clinicopathological factors.

Design: Tissue microarrays (TMAs) were constructed from FFPE tumor blocks from primary ductal breast carcinomas in 202 African-American females. Two separate 1 mm cores represented each case. Five micrometer sections were stained with mouse monoclonal antibody against HSP27 (Santa Cruz), HSP60 (Santa Cruz) and BCL2 (100/D5). The sections were evaluated for intensity of cytoplasmic and nuclear staining (1-3) and percentage of reactive cells; H-score was derived from the product of these measurements. Analysis was performed as a continuous variable. Bivariate analysis was done via $\chi 2$ analysis and survivability data was calculated via the generation of Kaplan-Meier curves (SPSS v19). Statistical significance was assumed if $p < 0.05$.

Results: HSP27, HSP60 and BCL2 expression was associated with ER+ ($p<0.001$), PR+ ($P<0.0001$), luminal subtypes ($p<0.001$), Her2 negative ($p<0.001$) and low grade ($p<0.001$) breast ductal cancers.

Conclusions: Our study finding of selective expression of HSP27, HSP60 and BCL2 in luminal subtypes breast ductal cancers in AA women suggests that antiapoptotic mechanisms predominate in the pathogenesis. HSP27 and HSP60 promote activation of nuclear factor-Kb (NF-kB), the key transcription factor for induction of survival genes in cells. Targeting HSP27, HSP60 and BCL2 activity may be effective as neoadjuvant therapy in luminal breast cancers.

193 Metabolomic Profiling Reveals Upregulated Fatty Acid Metabolism in Ductal Breast Cancers from African-American Women

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Background: Ductal
breast cancers (BCa), especially aggressive high grade triple negative breast cancers are more common in younger African-American patients when compared to other ethnic groups. Metabolomics, a study of cellular small-molecule metabolites, is undertaken. The goal is to identify metabolite markers in energy pathways that separate breast cancers from benign breast disease in African-American women.

Design: We used untargeted ^{1}H NMR metabolomics to identify common and unique metabolite markers in fibrocystic disease, fibroadenomas and breast cancer specimens. Fresh frozen tissues from 48 patients with median age of 21-78 years were analyzed by untargeted metabolomics analysis using ^{1}H nuclear magnetic resonance (NMR) spectroscopy. Multivariate and statistical analyses were used to determine the most significant metabolites that would differentiate the different BCa stages and grades from control mammoplasty tissues.

Results: In breast cancer patient samples, significant elevations of key fatty acid metabolism pathway markers including carnitine, choline, O-phosphocholine, O-acetylcholine and sn-Glycero-3-Phosphocholine are found when compared to fibrocystic disease and fibroadenoma ($p<0.05$).

Conclusions: Our study shows upregulated fatty acid metabolic pathway in all grades of ductal breast cancers when compared to benign breast changes and fibroadenoma. Carnitine transports long chain acyl fatty acids from cytoplasm to mitochondria that undergo beta fatty acid oxidation generating acetyl CoA that provides ATP via Krebs cycle. Phosphocholine and sn-Glycero-3-Phosphocholine are intermediates in lipid metabolism. Choline undergoes oxidation to trimethylglycine that acts as coenzyme in homocysteine-methionine pathway, ultimately affecting methylation of DNA. Our studies indicate fatty acid metabolism intermediates are involved in the development and progression of ductal breast cancers. Inhibiting fatty acid metabolism may be useful for treatment of ductal breast cancers.

194 Expression of GHRH-R, a Targetable Biopredictor, in Histologic Subtypes of Triple-Negative Breast Cancer

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Background: Growth Hormone Releasing Hormone (GHRH) has been shown to modify the growth behavior of many cancers, including breast. GHRH is produced by tumor cells, acts in an autocrine/paracrine manner, and requires the presence of GHRH receptor (GHRH-R) on the tumor cells to exert its effects. GHRH activity can be effectively blocked by newly developed antagonists of its receptor and hence, the expression of GHRH-R by tumor cells could serve as a predictor of response to GHRH-R antagonists therapy. In this study, we investigated the expression of GHRH-R in triple-negative breast cancers (TNBC), a group of tumors with no current standardized effective management. Since TNBCs are morphologically and immunophenotypically heterogenous, the results were correlated with the histologic subtypes of these tumors.

Design: Based on histomorphology and immunophenotype, 136 cases of primary TNBCs were further subdivided into medullary (HLA-DR-positive), metaplastic (P63-positive), apocrine (AR/GCDFP1-positive), lobular (E-Cadherin-negative) and invasive ductal carcinomas of no special type (NST). Immunohistochemistry for GHRH-R (AbCam) was performed on paraffin sections and the staining results were assessed semi-quantitatively as 0 (Negative), 1+ (low expression) 2+ (moderate) and 3+ (high expression). Fifty additional hormone and/or HER-2 receptor-positive breast cancers were used for comparison.

Results: Of the 136 TNBCs, 85 were classified as NST, 25 as metaplastic, 16 as medullary, 8 as apocrine, and 2 as lobular carcinoma. Overall, positive reaction for GHRH-R was seen in 77 (57%) of tumors including 77% of NST, 75% of apocrine and 100% of lobular carcinomas. All medullary carcinomas were negative for GHRH-R and, with the exception of one case with 1+ staining, none of the metaplastic carcinomas expressed GHRH-R ($p<0.005$). There was no significant difference between the NST and hormone-positive group with regards to the rate of GHRH-R expression ($p=0.36$).

Conclusions: The expression of Receptor for Growth Hormone Releasing Hormone varies considerably between histologic subtypes of triple-negative breast cancers. While most medullary and metaplastic carcinomas do not express GHRH-R, three-fourth of luminal type of triple-negative breast carcinomas show positive reaction. Testing for GHRH-R expression is therefore advisable if anti-GHRH-R therapy is being considered.

195 Accurate Diagnosis of Fibroepithelial Lesions of Breast on Core Needle Biopsy: A Multidisciplinary Approach

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Background: Fibroepithelial lesions (FEL) of the breast, i.e.fibroadenoma(FA) and phyllodes tumor(PT) can be difficult to distinguish on core needle biopsy(CNB). Overlapping morphologic features, particularly between a cellular FA and a benign PT may preclude a definitive diagnosis. In our institution, a diagnosis of FEL is made when the histomorphologic findings on CNB cannot reliably distinguish between these 2 entities. Most patients with this diagnosis are taken to surgery. Ultrasound imaging of PT may demonstrate a variable combination of increased vascularity, internal cyst/cleft formations, and echoheterogenicity of the lesion. In this retrospective study, we sought to evaluate the use of ultrasound imaging for further refining the diagnosis of FEL on CNB.

Design: All CNB diagnosed as FEL between 2013-2016 were retrieved from our institutional files. Subsequent excisions were reviewed and correlated with ultrasound findings. CNBs were reviewed for adequacy of sampling and the following morphologic features: stromal cellularity, overgrowth, atypia, heterogeneity, mitoses, and intra v. pericanalicular architecture. Ultrasound images were reviewed for the presence or absence of features typically ascribed to PT. The radiologic features of FELs ultimately diagnosed as PT were correlated with the histomorphologic findings present on CNB.

Results: We identified 85 CNB diagnosed as FEL. Of these, 40(47%) were found to be fibroadenomas, 36(42%) benign PT, 7 borderline PT and 2 malignant PT on subsequent excision. All cases of malignant and borderline PT and 26(72%) cases of benign PT had at least one of the characteristic radiologic features v. nine(22%) cases of fibroadenoma. The CNB cases associated with significant findings on ultrasound tended to have stromal overgrowth and stromal heterogeneity evident on 4 or more cores. When these morphologic features and radiologic findings were incorporated into the overall assessment of the FEL, a comment of "favor PT" or "favor FA" increased the diagnostic accuracy.

Conclusions: The distinction between PT and FA on CNB of breast may pose a diagnostic challenge due to overlapping morphologic features, resulting in a diagnosis of FEL. Surgical resection is the standard of care for a definitive diagnosis of PT on CNB. The diagnosis of FEL is indeterminate and may result in unnecessary surgery for some patients. A multidisciplinary approach including the input of pathologists and radiologists can further refine the diagnosis of FEL to help prevent the overtreatment of these patients.

196 *BRAF* Alterations in Metastatic Breast Cancer

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Background: *BRAF* alterations have been successfully targeted in a wide variety of malignancies, but have not been characterized in metastatic breast cancer (mBC).

Design: DNA was extracted from 40 microns of FFPE samples of 9159 mBC and comprehensive genomic profiling (CGP) was performed on hybridization-captured, adaptor ligation based libraries to a mean coverage depth of 579X for up to 315 cancer-related genes plus 37 introns from 14 genes frequently rearranged in cancer. Tumor mutational burden (TMB) was calculated from a minimum of 1.11 Mb of sequenced DNA and reported as mutations/Mb. Genomic alterations (GA) included base substitutions (SUB), INDELs, copy number alterations (CNA) and fusions/rearrangements.

Results: 115/9159 (1.2%) cases of BRAF altered mBC were identified. The mean age was 54.3 years (range 27 to 83 years). The primary tumor was used for CGP in 40 (35%) cases and from metastatic sites including lymph nodes, liver, bone, lung, brain adrenal and soft tissue in 75 (65%). 113 mBC were ductal including 1 inflammatory and 4 metaplastic carcinomas and 2 mBC were lobular mBC. Activating *BRAF* GA that may lead to aberrant MAPK signaling included amplifications (51.8%), V600E sub (15.7%), K601E sub (3.6%), other missense sub (21.6%), and fusions (6.0%); 3 additional mutations are uncharacterized for their effect on *BRAF* signaling activity (3.6%). The 7 identified fusions included *KIAA1549-BRAF* (4), *AGK-BRAF* (1), *FCHSD2-BRAF* (1), and *KLHDC10-BRAF* (1). There was a statistically significant reduction in *ERBB2* mutations in tumors harboring a *BRAF* GA (amplification or sub) (p=0.011). *ESR1* GA were identified in 7.8% of BRAF altered mBC. Of the 58 mBC harboring *BRAF* GA where ER/PR status was available, 55% were TNBC, 31% HR+/HER2-, 7% HR-/HER2+, 7% HR+/HER2+. The targetable GA most frequently amplified in mBC with *BRAF* GA, compared to *BRAF* WT mBC, included *CDK6* (p=0.001), *HGF* (p<0.001) and *MET* (p<0.001). The mean TMB of BRAF altered mBC was 7.5, the median was 15.3. The 23% frequency of *BRAF* mutated mBC with $\geq$ 10 mut/Mb was significantly greater than the 9% in *BRAF* WT mBC (p=0.0005).

Conclusions: Although representing only 1.1% of mBC cases, *BRAF* GA including both base substitutions and fusions are a potential new target for treatment of relapsed and aggressive disease. *BRAF* driven mBC is rarely HER2+, is most frequently encountered in triple negative disease and may be associated with higher TMB and clinical responsiveness to immunotherapies. Further study of *BRAF* mutation positive breast cancer in the setting of basket clinical trials of existing and novel BRAF and MEK inhibitors appears warranted.

197 Validation of Prosigna (Breast Cancer Prognostication Assay) & Its Concordance with Oncotype DX & Mammoprint: Geisinger Health System's Experience

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Background: Prosigna (P) is an FDA approved *in vitro* diagnostic assay which determines breast cancer prognostic gene signature of 58 genes in FFPE breast tissue harboring hormone receptor (+) invasive breast carcinoma (IBR), while reporting recurrence risk scores (RSs) in 3 tiers (low:L, intermediate:Int, high:H). Oncotype DX (O) is a non-FDA approved assay interrogating 21 genes in a 3 tier system, with Int group larger than that of the P. Mammoprint (M) is an FDA approved test reporting 70 genes in a 2 tiered manner (L, H), offering clinical utility especially in Int group in regards to who should receive chemotherapy (CT; if H by M) or hormonal therapy (if L by M).

Design: This study validating P, with confirmations done in an outside lab: Fox Chase Cancer Center (FC), also analyzed data from training runs, and compared P to O & M results. RNA extracted from FFPE tissue harboring IBC was analyzed by NanoString nCounter® Dx Analysis System to digitally detect multiple RNA targets providing prognosis, predicting therapy and assessing 10 year distant recurrence risk.

Results: RNA controls (L, H) run in duplicate for 3 days (Ds) by 2 med techs (MTs) received Mean+/-SD of 29.2+/-0.6 (2.1% CV) and 71.8+/-0.9 (1.2% CV), respectively. 10 FFPE IBC samples analyzed by 2 MTs on 2 Ds in triplicate, starting from macrodissection rather than RNA, provided reproducible RSs (0%-5% CV). P results confirmed by FC showed 100% concordance (0%-4.6% CV). 4 MTs' training runs of 3 samples tested in duplicate on 2 Ds resulted in 100% concordance across 3 tiers (1%-7.3% CV). P results when compared to those of O were concordant in only n=5 (3 L, 2 H), with discordance rate being 50%: 3 L by P but Int by O; 1 Int by P but H by O (also L by M) & 1 L by P but H by O. 28 clinical samples tested by P (L:13, Int:9, H:6) had a TAT of 1 wk. Of those scored Int by P, all 7 were L by M.

Conclusions: Prosigna is highly reproducible and accurate, displaying high concordance rates with O in H & L groups, and contracting Int group with subsequent improvements in therapy decisions & patient/provider satisfactions. P's decentralized testing ability offers improved TAT. M testing is beneficial in Int P scores as low M scores may decrease patients who may otherwise receive CT. This study using macrodissected FFPE tissue as starting material in repeat testing is likely the 1st to investigate clinical utility of M and P in Int group.

198 Size and Heterologous Elements Predict Metastases in Malignant Phyllodes Tumors of the Breast

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Background: Breast phyllodes tumors (PTs) are uncommon fibroepithelial neoplasms comprising 0.3 - 1.0% of primary breast neoplasms. Majority are benign, carry a good prognosis, and are generally well managed with surgery. However, malignant PTs and occasionally borderline PTs can behave in an aggressive manner with local recurrence rates of malignant PTs ranging from 15 to 40%, with distant metastasis rates of 9 to 27%. The prognosis of patients with metastasis is poor as many are unresponsive to chemotherapy and risk of death is high.

Design: PTs diagnosed at the Department of Pathology, Singapore General Hospital, from 1994 to 2015 were reviewed. Follow up data was obtained from case records. Metastasis free (MFS) and overall survivals (OS) were calculated as the duration from diagnosis to metastasis or death respectively. Cases that were lost to follow-up were censored. Sites of metastasis were also recorded. Survival outcomes were estimated with the Kaplan–Meier method and compared between groups using log-rank statistics. Cox regression was carried out to identify factors predictive for metastasis. Two-sided statistical significance level was set at 0.05.

Results: Among 952 PTs diagnosed during the study period, 83 (8.7%) were malignant which formed the study group. Mean, median age was 48 years, range 21 to 71 years. Tumor size measured 30 to 220 mm (mean 90mm, median 77mm). Follow-up data was available for 68 patients. Mean, median follow-up was 90 and 57 months with a maximum of 291 months. Metastasis occurred in 16 of 68 patients (23.5%) with follow-up information. Metastatic sites were lung (12 patients), lung and liver (1 patient), lung and brain (1 patient), soft tissue (1 patient), spine (1 patient). Malignant heterologous elements were recorded in 16 (23.5%) tumors. Individual clinicopathological parameters had no impact on outcome. On Kaplan-Meier analysis women with large tumors and presence of malignant heterologous elements showed trends for poorer MFS (p=0.217 and p=0.566 respectively). However, the combination of large tumors (>9cm) containing malignant heterologous elements disclosed significantly worse MFS (p=0.043) and a trend for poorer OS (p=0.238). On multivariate analysis, large tumors harboring malignant heterologous elements independently predicted metastasis (95%CI 1.041-12.517, HR 2.434, p=0.049).

Conclusions: Size and malignant heterologous elements predicted metastasis in malignant PTs. Further work needs to be done in determining if protein biomarkers and genomic aberrations are able to further refine the metastatic risk and offer therapeutic targets.

199 Genomic Profiling of Secretory Carcinoma

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Background: Secretory carcinoma (SC) is a rare breast cancer (BC) subtype with ETV6-NTRK3 fusion. By immunohistochemistry (IHC), SC belong to the basal-like spectrum and most are triple negative (TN), but genomic profiles of SC have not been described and whether SC share genetic features of other basal/TNBC is unknown. Aside from ETV6-NTRK3, the relatedness of SC molecular profile to mammary analog SC (MASC) in other organs is also unknown. We profiled SC using capture-based next generation sequencing (NGS) and compared the results to salivary gland MASC and data of basal BC in order to gain insight into the biology of these rare tumors.

Design: DNA was extracted from 9 SC, 6 MASC and matched normal tissue. NGS was performed targeting exons of 510 cancer genes and 40 introns. Duplicate reads were removed computationally for allele frequency determination and copy number(CN) calling. Single nucleotide variants, insertions/deletions and copy number alterations(CNA) were evaluated. Fluorescence in situ hybridization(FISH) was performed with ETV6 split-apart probe. IHC was performed for mammaglobin(MG), S100, SOX10 and MUC4.

Results: By FISH, ETV6 translocation was identified in all SC (n=9) and available MASC (n=4). By NGS, ETV6-NTRK3 was identified in all SC and 5/6 MASC, with a novel ETV6 fusion identified in ETV6-NTRK3 negative MASC. The variant MASC was SOX10 and MUC4 negative, in contrast to all other SC and MASC; all tumors expressed MG and S100. The mutation burden in all cases was low (0-3 genes on the panel). No other pathogenic mutations were identified in any tumors. All cases had simple genomes with few CNA; 5(56%) SC and 2(33%) MASC showed no CNA.

		ETV6 FISH	ETV6-NTRK3 fusion	Alternate ETV6 fusion	Gain	Loss
SC	1	+	+	-	8q	-
	2	+	+	-	16	interstitial 5q
	3	+	+	-	8,distal 12p	distal 15q
	4	+	+	-	13,16	-
	5	+	+	-	-	-
	5(DCIS)	+	+	-	-	-
	6	+	+	-	-	-
	7	+	+	-	-	-
	8	+	+	-	-	-
	9	+	+	-	-	-
MASC	1	+	+	-	16,20	18
	2	+	+	-	distal 12p,15	-
	3	+	+	-	-	-
	4	NA	+	-	interstitial 5q,7	proximal 15q,22
	5	+	-	+	-	-
	6	NA	+	-	-	14,22

Conclusions: SC do not harbor mutations common in other basal BC. SC and MASC show similar genomic features, including low mutation burden and simple CN profile. Aside from fusion genes, no other pathogenic mutations were identified in SC or MASC. MASC with variant ETV6 fusion has similar morphology but different immunoprofile than SC or MASC with ETV6-NTRK3 and may represent a distinct subset.

200 Genomic Profiling of Synchronous Bilateral Lobular Carcinoma

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Background: Invasive lobular carcinomas (ILC) are more often bilateral than other breast cancers. Genomic studies have uncovered pathogenic pathways in ILC, but synchronous bilateral ILC (sbILC) have not been similarly analyzed, and the genomics of these tumors and reasons underlying bilaterality are unknown. We used capture-based next generation sequencing (NGS) to profile sbILC to gain insight into pathogenesis of these tumors.

Design: DNA was extracted from 14 sbILC in 7 patients and matched normal tissue. NGS was performed targeting coding regions of 510 cancer genes and 40 introns. Duplicate sequence reads were removed computationally for accurate allele frequency determination and copy number calling. Single nucleotide variants, insertions/deletions, and copy number alterations (CNA) were evaluated.

Results: Mean age was 64 (range 50-82). Breast cancer family history was noted in 4/6. All patients had bilateral LCIS, often extensive and multifocal, and 4/7 had DCIS, which was bilateral in 2. Recurrently mutated genes in ILC included PIK3CA (7/14), PIK3R1 (2/14), MAP3K1 (2/14), RUNX1 (3/14), CBFB (2/14), TBX3 (2/14) and CDH1 (11/14). All patients had PI-3 kinase pathway (FGFR2, PIK3CA, PIK3R1, PTEN) mutations in at least 1 tumor and 4 were bilateral. PIK3CA mutations were bilateral in 2 patients; 2 others with PIK3CA mutations had contralateral PIK3R1 mutations. Mutations in the RUNX1/CBFB dimer were present in 4 patients; 1 patient with RUNX1 mutation had contralateral CBFB mutation. No pathogenic germline variants were identified.

sbILC; Right(R), Left(L)	PI-3 kinase	MAP kinase	RUNX1/ CBFB	TBX3	CDH1	1q+,16q-
1R	PIK3CA N345K		RUNX1 Q397fs		F375fs	y
L						y
2R	PIK3CA H1047R		RUNX1 S265fs		E648*	y
L	PIK3CA H1047L				E813*	y
3R	PIK3CA H1047R		RUNX1 H404fs		Q23*	y
L	PIK3CA H1047R		CBFB R33fs		765_765del	y
4R	PIK3CA N345K				T38fs	y
L	PIK3R1 438_439del		CBFB c.117_118TT		A592fs	y
5R	PTEN V343fs	MAP3K1 T911fs, V1105fs			c.2164 +2TAAGT >AGAG	y
L				S266fs		n
6R	PIK3CA H1047R	MAP3K1 M1442V, P1474L			W156*	y
L	PIK3R1 570_574del			287_287del		n
7R		MAP2K4 G294E			T378fs	y
L	FGFR2 N549K				Q23*	y

Conclusions: SbILC share commonly mutated pathways with ILC. SbILC show alternate genetic mechanisms to modulate signaling pathways common to both tumors, highlighting the pathogenetic importance of these pathways. Pathogenic germline variants were not identified.

201 Fibromatosis of the Breast: Diagnostic Accuracy of Core Needle Biopsy

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Background: Fibromatosis of the breast is a rare neoplasm that mimics benign and malignant entities. Treatment has traditionally been surgical excision with current trends towards conservative management. Observation after diagnosis by core needle biopsy (CNB) can be an option. The aim of this study was to evaluate the accuracy of CNB for diagnosing fibromatosis.

Design: The pathology database was searched over the period of 1997 to 2016 for cases in which fibromatosis had been diagnosed, or included in the differential diagnosis, on either a CNB or excision or both. Only lesions located in the breast or breast/anterior chest wall were included. Morphologic and immunohistochemical (IHC) results were reviewed.

Results: A total of 29 cases were identified. Twenty-three cases of fibromatosis on excision had prior CNB diagnoses of fibromatosis (n=11, 48%), spindle cell lesion with a differential diagnosis that included fibromatosis (n=8, 35%), scar (n=2), dense stroma (n=1), and nodular fasciitis (n=1). Six cases had a diagnosis of fibromatosis, or included fibromatosis in the differential diagnosis, but had a different diagnosis on excision: phyllodes tumor (n=2, low and intermediate grade), unclassified myofibroblastic proliferation (n=1), fibroadenomatoid change (n=1), sclerosing papilloma (n=1), and scar (n=1).

Positive nuclear immunoreactivity for β-catenin and the absence of staining for CD34 were the most helpful studies to diagnose fibromatosis. Of the 12 cases in which fibromatosis was not definitively diagnosed on CNB, both were performed in only 2 cases. Of the 6 cases in which fibromatosis was incorrectly diagnosed on CNB, these 2 studies were performed in 3 cases, but were atypical for fibromatosis in 2 of the 3. Of the 13 cases with lacking optimal IHC, about half were either consults without blocks or older cases prior to the common use of β-catenin. All cases tested for keratin (n=16) or p63 (n=13) were negative.

Conclusions: An accurate and definitive diagnosis of fibromatosis was made on CNB in almost half of cases and was suggested in another third. More frequent use of IHC would likely have resulted in a greater number of definitive diagnoses. In addition, misdiagnosis of fibromatosis on CNB would likely be avoided by greater use of IHC, as well as attention to patterns not typical for fibromatosis. Fibromatosis was most commonly mistaken for other benign stromal lesions. CNB can be an accurate method of diagnosing fibromatosis, allowing conservative management with observation for a select group of patients.

202 Breast Cancer in Li-Fraumeni Syndrome: Morphologic Evaluation of Invasive and In Situ Carcinomas

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Background: Breast cancer is one of the most common tumors among patients with Li-Fraumeni syndrome (LFS), a rare autosomal dominant hereditary syndrome characterized by germline TP53 mutations. The immunophenotypic profile of these tumors has recently been described; however, the morphologic features of invasive and in situ carcinoma arising in this setting have not been studied in detail.

Design: Twenty-four cases of invasive and in situ breast cancer from 23 patients with a known TP53 germline mutation were reviewed for morphologic features, including architecture, nuclear grade, mitotic rate, presence of necrosis and associated lymphocytic infiltrate.

Results: There were 15 cases of invasive carcinoma (IC) and 9 cases with only ductal carcinoma in situ (DCIS), 2 of which included microinvasion. Median age of presentation was 34 for DCIS and 31 for IC. The majority of the cases, including both ICs and DCIS, were HER2 positive (14/23, 61%). Invasive carcinomas were most often ductal/NST (93%), with only 1 case showing mixed ductal and lobular features. Eleven of the ICs showed high nuclear grade (75%) and the majority were classified as high grade (60%) by modified Scarff-Bloom-Richardson (mSBR) grading . Stromal tumor infiltrating lymphocytes (TILs) ranged from <1-50% (median=5%). In cases with DCIS only, high nuclear grade was observed in 8/9 cases (89%), with central necrosis in 7/9 cases (78%), solid architecture (100%) and periductal stromal/lymphocytic response in 7/9 cases (78%). DCIS seen in association with ICs showed high nuclear grade in 8/11 cases (73%), with central necrosis in 7/11 cases (64%) and stromal/lymphocytic response in 5/11 cases (45%).

Conclusions: Invasive carcinomas associated with TP53 germline mutations are most often ductal with high mSBR grade and high-grade nuclear features. When DCIS is diagnosed alone, it is usually solid type, high nuclear grade with central necrosis and periductal lymphocytic response. Most invasive carcinomas showed a range of TILs, but none showed an extensive infiltrate (>50%). The pathologic features of breast cancer associated with Li-Fraumeni syndrome are distinct from other germline-associated breast cancers.

203 Assessment of Cell Density and Ki67 in Initial, Intermediate CNB and in Resection Specimen After Neoadjuvant Chemotherapy

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Background: Ki 67 index as well as cell distribution and cell density are the main characteristic for tumor pathology especially in neoadjuvant settings. We performed image analysis tool to characterize Ki67 and cell density within tumor before, during and after neoadjuvant therapy

Design: The study included 51 breast carcinomas before, during and after neoadjuvant therapy. We have analyzed Ki67 positive cells and total number of tumor cells in breast cancer from a 1 sq mm sample of histology slide before, during and after NCT using whole slide scanning and image analysis tools.

Results: Mean absolute tumor cells in 1 mm2 of histology slide in first sample of breast cancer before neoadjuvant therapy was 7243,71/mm2, Ki67 level was 46,86%. In intermediate CNB after 2 - 3 cycles of neoadjuvant therapy we had 15 cases with no tumor cells in intermediate biopsy and we assessed that cases as no adequate samples. Cell density in rest CNB comes down to 4704,42/ mm2 and Ki67 was also decreased to 22, 64% (p=0,000791).. In final resection specimen there was 9 cases (17,64%) with pCR. And there was different level of cell density reduction from 4052/mm2 (no response), Assessment of cell density in initial, intermediate CNB and in resection specimen after

neoadjuvant chemotherapy Ki67 was 27,8% with no significant differs between intermediate and resection specimens to 432/mm2 in tumor bed (complete response) according Miller and Payne grading system.

Conclusions: image analysis is a good and objective tool to determinate cell density and Ki67 level in histology slides in neoadjuvant settings

204 Expression of Interleukin-13 Receptor Alpha 1 Correlates with HER2 Status and Survival of Patients with Invasive Breast Cancer

Hee Jung Kwon, Byung-Heon Lee, Young Kyung Bae. Yeungnam University College of Medicine, Daegu, Republic of Korea; School of Medicine, Kyungpook National University, Daegu, Republic of Korea.

Background: Interleukin-13 (IL-13) is an immunoregulatory and anti-inflammatory cytokine produced by numerous immune cells. Plasma membrane receptor for IL-13 (IL-13R) has known to be expressed on various human malignancies, as well as immune cells.

Design: We evaluated expression of IL-13R alpha 1 (IL-13Rα1), one of IL-13R subtypes, by immunohistochemistryin tissue microarrays of 1,213 invasive breast cancer (IBC) samples to clarify the prognostic significance of IL-13Rα1 expression.

Results: High IL-13Rα1 expression was observed in 619(51%) cases. IL-13Rα1 expression was associated with older age (P=0.022), lymph node metastasis (P=0.015), ductal and micropapillary histologic subtypes (P<0.001), lymphovascular invasion (P=0.012), HER2 positivity (P<0.001), and high Ki-67 index (P=0.039). No significant correlation was found between IL-13Rα1 expression and clinicopathological variables including tumor size, histologic grade, hormone receptors, and tumor-infiltrating lymphocytes levels. Patients with high IL-13Rα1 expression showed worse overall survival (P=0.044) and disease-free survival (DFS, P=0.002) than those with low/negative expression of IL-13Rα1. In the subgroup analysis, a correlation between IL-13Rα1 expression and survival was observed in HER2-negative, but not in HER2-positive tumors. High expression of IL-13Rα1 was found to be an independent prognostic factor for DFS in multivariate analysis (P=0.016).

Conclusions: Our results suggest that IL-13 and IL-13R interaction promotes cancer cell growth and survival. In addition, IL-13Rα1 expression could be a promising prognostic marker in patients with IBC.

205 Correlation Between the Expression of CEACAM6 and Clinicopathological Variables in Breast Cancer

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Background: CEACAM6 is frequently overexpressed in adenocarcinomas from various organs such as lung, colon, and breast. It is also a novel candidate for a new therapeutic target using monoclonal antibodies. We assayed the expression of CEACAM6 and the correlation with clinicopathological variables in breast cancer.

Design: Immunohiostochemical assay for CEACAM6, using 8F5 antibody, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), p53, and ki-67 was performed in 177 invasive breast cancer specimens. Clinical variables, such as patients' ages, nuclear and histological grades, pathological stages, lymphovascular and perineural invasions, were also evaluated.

Results: Overexpression of CEACAM6 was observed in 95 cases (53.7%). It was associated with hormone receptor (HR)-positive (either ER- or PR-positive, p=0.033), negative p53 overexpression (p=0.016) and low ki-67 indices (p=0.045). After dividing into HR-positive and HR-negative groups, CEACAM6 was associated with positive HER2 expression (p=0.007) and low ki-67 index (p=0.005) in HR-negative group whereas no variables were associated in HR-positive group.

Conclusions: Various subtypes of breast cancer, including luminal and HER2-positive subtypes, can be a therapeutic target for CEACAM6.

206 "Inside-Out" p120 Immunostaining Pattern in Invasive Micropapillary Carcinoma of Breast; Another Unequivocal Evidence of Reversed Polarity

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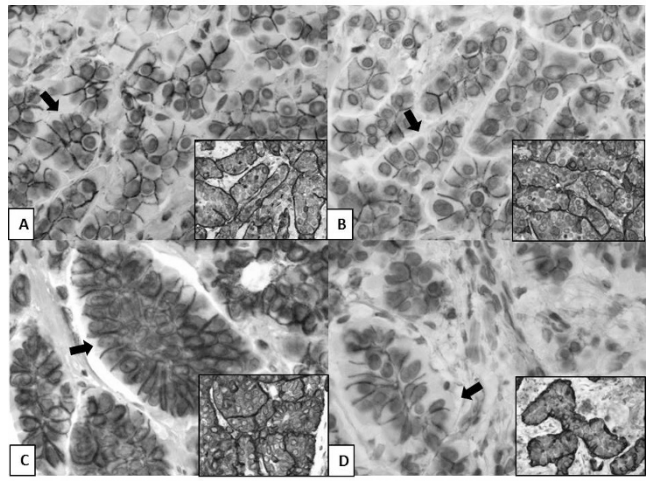
Background: By immunohistochemistry(IHC), p120 catenin(p120) has a distinct membranous expression in the luminal epithelium of terminal duct lobular unit. p120 IHC expression is limited to lateral cell borders; luminal surface is p120 negative. Tumor cells of invasive micropapillary carcinoma(IMPC) of breast show reversed cell polarity with characteristic "inside-out" pattern of tumor cell membrane. p120 expression pattern in IMPC is not known. We hypothesized that reversed cell polarity seen in IMPC can be identified by p120 IHC.

Design: Consecutive invasive breast cancers from 01/2014-07/2015 diagnosed as IMPC or with "retraction clefts" were identified. H&E slides were reviewed (KS & MRQ) and cases with histological features of IMPC were identified. The IMPC proportion in a tumor was measured on a semi-quantitative scale: <25%, 26-50%, 51-75% and >75%. p120(monoclonal;98/pp120;Biocare Medical) and EMA(monoclonal;E29;Dako) IHC was performed (Dako Platform) on a representative tumor tissue block. p120 IHC pattern in IMPC was compared to non-IMPC foci.

Results: A total of 43 cases qualified for study: 20(46.5%) tumors with IMPC component & 23(52.5%) invasive carcinoma of no special type(IDC) with retraction clefts. Four of 20(20%) cases with IMPC component were pure IMPC (>75% IMPC); 5/23 with <25%, 1 with 26-50% & 10 with 51-75% IMPC component. Two of 20 IMPC were mucinous carcinoma. EMA IHC revealed "inside out" pattern of immunostaining, confirming histological diagnosis in all 20 cases. Distinct p120 membranous expression pattern was noted in IMPC: granular/continuous positivity limited to lateral cell borders with no staining of membrane facing the stroma. p120 staining recapitulated luminal epithelium with reversed polarity. IDC cases and non-micropapillary components of IMPC showed circumferential granular or continuous membranous staining with rare focal loss of staining towards stromal surface.

Figure 1 p120 IHC on 4 IMPC cases(A-D) showing "inside-out" staining pattern with absent p120 in the membrane facing stroma(arrow). Inset is corresponding EMA IHC for each case.



Conclusions: We describe a previously unreported distinct p120 staining pattern in IMPC. p120 IHC can be employed to support IMPC diagnosis in a morphologically equivocal case. Further studies are needed to evaluate the prognostic significance of p120 expression in IMPC.

207 The Genomic Landscape of PALB2-Associated Breast Cancers

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Background: Partner and localizer of BRCA2 (PALB2) is a key protein that interacts with BRCA1/2for homologous recombination (HR) repair and tumor suppression. *PALB2* germline mutations have been identified to be causative of hereditary breast cancer. Here we sought to define the repertoire of somatic genetic alterations in PALB2-associated breast cancers, and to investigate whetherPALB2-associated breast cancers display features of homologous recombination deficiency.

Design: Invasive ductal carcinomas of no special type from *PALB2* germline mutation carriers were microdissected. Matched tumor and normal DNA were subjected to a combination of whole-exome (n=14) or targeted capture (n=6) massively parallel sequencing (MPS) with a sequencing assay targeting the entire coding region of 410 genes and regulatory and intronic regions of selected genes. Somatic mutations, insertions/ deletions, copy number alterations, large-scale state transitions (LSTs) and mutational signatures were detected using state-of-the-art bioinformatics algorithms.

Results: MPS analysis yielded a median of depth of 118x (range 33x-193x) and 232x (range 73x-1168x) for whole-exome and targeted capture MPS, respectively. The median number of non-synonymous mutations was 56 (range 28-61) by whole-exome and 9 (range 3-17) by targeted capture MPS. The genes most frequently affected by non-synonymous somatic mutations were *PALB2* (25%), *TP53* (20%), *CTNNA2* (15%), *CNTNAP4* (15%), *PIK3CA* (15%) *and NOTCH3* (15%). Twelve cases displayed *PALB2* bi-allelic inactivation; in seven, the second hit was in the form of somatic loss of the *PALB2* wild-type allele, and in five, the second hit was in the form of *PALB2* somatic mutations (two truncating, three frameshift). A significant association between LSTs and *PALB2* bi-allelic inactivation was observed (*P*=0.035), with high LST scores found in 83% of cases harboring *PALB2* bi-allelic inactivation. The mutational signature 3 (BRCA1/2 signature) was found only in breast cancers with *PALB2* bi-allelic inactivation.

Conclusions: A subset of PALB2breast cancers display genomic features consistent with HR-deficiency, and the majority of these cases display *PALB2* bi-allelic inactivation. PALB2-associated breast cancers show a heterogeneous repertoire of somatic genetic alterations, including the mechanism of loss of the wild-type allele of *PALB2*.

208 Triple Negative Breast Cancer Has Worse Overall Survival and Cause-Specific Survival Than Non-Triple Negative Breast Cancer

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Background: The current American Joint Committee on Cancer (AJCC) staging manual uses tumor size, lymph node and metastatic status to stage breast cancer across different subtypes. We examined the prognosis of triple negative breast cancer (TNBC) vs non-TNBC within the same stages and sub-stages to evaluate whether TNBC had worse prognosis than non-TNBC.

Design: We reviewed the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) data and identified158,358 patients diagnosed with breast cancer from 2010 to 2012. The overall survival (OS) time and breast cancer cause-specific survival time were compared between patients with TNBC and non-TNBC in each stage and sub-stages. The results were validated using a dataset of 2049 patients with longer follow-up from our institution.

Results: Compared with patients with non-TNBC, Patients with TNBC had worse OS and breast cancer cause-specific survival time in every stage and sub-stage in univariate and multivariate analyses adjusting for age, race, tumor grade and surgery and radiation treatments in the SEER data. The worse OS time in patients with TNBC was validated in our institutional dataset.

Conclusions: Patients with TNBC have worse survival than patients with non-TNBC. The new AJCC staging manual should consider breast cancer biomarker information.

209 Prognostic Significance of PD-1/PDL-1 Expression in Breast Cancer and Tumor Associated Immune Cells

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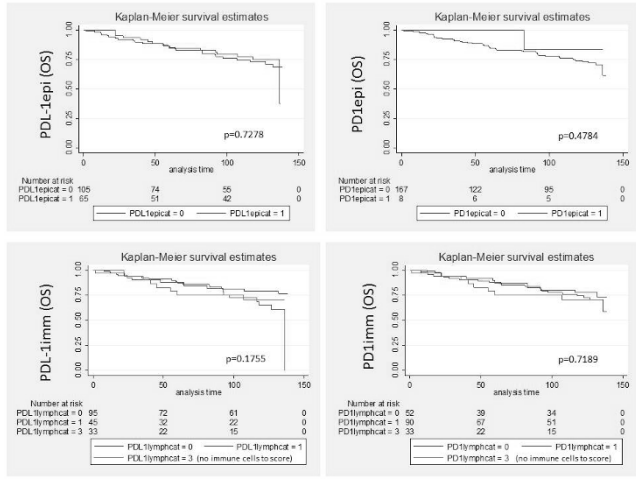
Background: The interaction between Programmed death 1 (PD-1) and its ligands PDL-1/2 within tumor microenvironment helps tumor evade T-cell cytotoxicity. Drugs targeting this immune check point are variably effective in different tumor types and in lung cancer therapeutic effect appears to be associated with diffuse-strong immunoreactivity for PDL-1 in the tumor cells.

Design: PD-1 and PDL-1 expression was examined in 198 consecutive invasive breast carcinomas represented on tissue microarray. Expression for both PD-1 and PDL-1 within epithelium (epi) and immune cells (imm) was examined. Due to only weak reactivity in most cases, an H-score of 1 was considered a positive result. Prognostic significance of PD-1epi, PD-1imm, PDL-1epi, and PDL-1imm immunohistochemical expression is reported with respect to disease free (DFS) and overall survival (OS).

Results: PDL-1 expression is membranous in tumor cells. PDL-1 and PD-1 expression in immune cells and PD-1 expression in epithelium is cytoplasmic and membranous. Overall, the expression for all markers was weak and focal/patchy. The expression pattern with respect to ER status is shown below.

	ER+	ER-	p-value
PD-L1epi+	51/138 (40%) Mean H-score 39	14/32 (44%) Mean H-score 90	0.5461
PD-L1imm+	27/109 (25%) Mean H-score 69	18/31 (58%) Mean H-score 93	0.0009
PD1epi+	7/143 (5%) Mean H-score 77	1/32 (3%) H-score 230	1.0
PD1imm+	66/111 (59%) Mean H-score 44	24/31 (77%) Mean H-score 90	0.0911

PD-1 and PDL-1 expression in immune cells is more frequent in ER-neg tumors. Less than 10% cases showed ≥50% tumor cell positivity with PDL-1 (a requirement to receive pembrolizumab in lung cancer) and only 1% of the cases showed diffuse strong reactivity. There was no difference in DFS and OS based on PD-1 and PDL-1 positivity.



When data was examined separately for ER+ and ER-neg tumors, the results remained similar except for slightly worse OS for cases with PDL-1+ immune cells (p=0.0312) in ER+ tumors.

Conclusions: PD-1 and PDL-1 expression is generally not prognostic in breast cancer. The often negative or weak IHC expression for PD-1 and PDL-1 suggests limited utility of immune check point inhibitors in breast cancer.

210 PDL-1 (Clone 28-8 and Clone 22C) Expression in HER2+ Breast Carcinoma: Correlation with Tumor Infiltrating Lymphocytes and Pathologic Variables

Yanchun Li, Hossam Jabbour, Xuan Peng, Song Yao, Mateusz Opyrchal, Li Yan, Thaer Khoury. Roswell Park Cancer Institute, Buffalo, NY.

Background: Recent studies in multiple epithelial cancers including breast carcinoma (BC) have shown programmed death ligand 1 (PDL-1) is expressed on tumor cells. However, little is known about the staining patterns, its expression in HER2+ BC, and the relationship with the degree of stromal tumor infiltrating lymphocytes (Str-TILs) and other pathologic variables.

Design: HER2+ (n=264) BC cases were collected between 1996 and 2013. Tumor tissue was constructed in tissue microarray (TMA) in triplicate. At least a single full section of Hematoxylin and Eosin stained slide was reviewed by two pathologists to score Str-TILs following the current guidelines. TMA slides (n=7) were stained with two clones of anti- PDL-1 antibodies (28-8 and 22C) and scored by two pathologists as well. Three staining patterns were recognized: tumor-membranous, tumor-nuclear, and in TILs. All scores were averaged between the two scorers.

Results: Positive staining for 28-8-membranous, 28-8-nuclear and 28-8-TILs was identified in 23%, 17.6%, and 33% of cases, respectively. Positive staining for 22C-membranous, 22C-nuclear and 22C-TILs was identified in 13%, 10.2%, and 20.4% of cases, respectively. Str-TILs median (range) was 25% (0% to 85%). Cases with Str-TILs>15% had higher expression of 28-8-membranous with interquartile range (IQR) of 2.5 (0 to 255) vs. 0 (0 to 26.67) for low 28-8-membranous ($p<0.001$). Cases with Str-TILs>15% had higher expression of 28-8-TILs with IQR of 15.6 (0 to 133) vs. 0.8 (0 to 23.3) for low 28-8 TILs ($p<0.001$). Cases with Str-TILs>15% had higher expression of 22C-membranous with IQR of 0.83 (0 to 220) vs. 0 (0 to 5) for low 22C-membranous ($p<0.001$). Cases with Str-TILs>15% had higher expression of 22C-TILs with IQR of 4.17 (0 to 66.67) vs. 0 (0 to 20) for low 22C-TILs ($p<0.001$). Nuclear staining (clone 28-8 or 22C) did not correlate with Str-TILs. 28-8-membranous correlated with higher Nottingham grade [IQR 1.67 (0 to 255) for grade III vs. 0 (0 to 41.67) for grade II vs. 0 (0 to 0) for grade I, p=0.002]. 28-8-membranous had higher expression in HER2-like vs. luminal-B-like with IQR of 1.67 (0 to 255) vs. 0.42 (0 to 213.3), respectively, p=0.019].

Conclusions: Anti-PDL-1 antibody (clone 28-8) had higher positivity than clone 22C. PDL-1 expression (both clones) staining in tumor membrane and in TILs, but not in tumor nucleus had strong correlation with Str-TILs. There was variable correlation for PDL-1 with the pathologic variables depending upon the clone used. Membranous 28-8 correlated with higher Nottingham grade and HER2-like subtype.

211 PDL-1 (Clone 22C) Stromal Expression Predicts Better Overall Survival in HER2+ Breast Carcinoma

Yanchun Li, Hossam Jabbour, Xuan Peng, Song Yao, Mateusz Opyrchal, Li Yan, Thaer Khoury. Roswell Park Cancer Institute, Buffalo, NY.

Background: PD-1/PDL-1 axis has emerged as a promising new therapeutic target for cancer. Tumor PDL-1 protein expression may predict response to drugs targeting this pathway. The purpose of the study was to investigate PDL-1 expression in HER2+ breast carcinoma (BC) and correlate with the clinical outcome.

Design: HER2 positive (n=264) BC cases were collected between 1996 and 2013. Tumor tissue was constructed in tissue microarray (TMA) in triplicate. Key clinicopathological information collected include patient's age, histologic subtype, Nottingham grade, tumor size, lymph node status, TNM-stage, ER and PR status, therapy modality including chemotherapy (with or without trastuzumab), hormonal therapy, and radiation therapy; and clinical outcomes including disease free survival (DFS) and overall survival (OS). The tumors were subclassified as luminal-B-like (ER+ and/or PR+) or HER2-like (ER- and PR-). TMA slides (n=7) were stained with two clones of PDL-1 antibodies (28-8 and 22C) and scored by two pathologists. Three staining patterns were recognized: tumor-membranous, tumor-nuclear, and in TILs. All scores were averaged between the two scorers.

Results: Positive staining for 28-8-membranous, 28-8-TILs, 22C-membranous, and 22C-TILs was identified in 59 (23%), 85 (33%), 29 (13%), and 42 (20.4%) of the cases, respectively. In addition to age, tumor size, node status, TNM-stage, chemotherapy, and trastuzumab therapy, these variables predicted the OS including 28-8-membranous (p=0.042), 28-8-TILs (p=0.042), and 22C-membranous (p=0.027). 22C-TILs had borderline significance (p=0.078). In multivariate analysis, 22C-TILs was the only significant variable with hazard ratio (HR) of 0.28 (95% CI 0.11 to 0.74) (p=0.0096) when all types of chemotherapies were included and remained significant when trastuzumab was combined with chemotherapy (HR=0.35, 95% CI 0.14 to 0.86, p=0.021). For DFS the following variables were statistically significant in univariate analysis including tumor size (p=0.037), node status (p=0.032) and 28-8-TILs (p=0.011). In multivariate analysis, 28-8-TILs became non-significant. The data was then stratified into luminal-B-like and HER2-like. None of the PDL-1 variables was statistically significant in multivariate analysis of DFS or OS. Similar stratified analyses were conducted in trastuzumab-treated and trastuzumab-non-treated. Again, none of the PDL-1 variables was significant.

Conclusions: Stromal PDL-1 detected by C22 antibody predicted better OS in HER2+ BC, regardless of trastuzumab use. PDL-1 did not differentially predict DFS or OS in HER2+ BC subgroups.

212 HER2 Intratumoral Heterogeneity Is Associated with Incomplete Response to Anti-HER2 Neoadjuvant Chemotherapy in HER2-Positive Breast Carcinoma

Zaibo Li, Hiroaki Nitta, Yanjun Hou, Peter Banks. Ohio State University, Columbus, OH; Ventana Medical Systems, Inc, Tucson, AZ.

Background: Anti-HER2 targeted reagents have been widely used in HER2-positive breast cancer patients. In spite of their clinical benefit, resistance to these reagents has been identified in up to 50% of patients. HER2 intratumoral heterogeneity, described as the coexistence of multiple tumor cell subpopulations with distinct HER2 amplification characteristics within the same tumor, has been reported in up to 40% of breast cancers. However, HER2 intratumoral heterogeneity has not been well studied as a potential factor in the resistance to anti-HER2 reagents.

Design: 64 HER2-positive invasive breast carcinoma patients treated with anti-HER2 neoadjuvant chemotherapy and follow-up resection were included in this study. HER2 gene-protein assay (GPA), combining immunohistochemistry (IHC) and dual *in situ* hybridization (ISH), was performed on pre-treatment core-needle biopsies. HER2 intratumoral heterogeneity was defined as any of the following: genetic heterogeneity (clustered or intermixed) and microheterogeneity (clustered or scattered tumor cells with HER2 gene amplification on ISH but negative IHC). We defined pathologic complete response (pCR) as no residual invasive tumor (excluding DCIS) and no lymph node metastases; and incomplete response as presence of residual invasive tumor in breast or lymph node metastasis.

Results: 39 patients had pCR and 25 patients had incomplete response. There was no significant difference in age, Nottingham grade or nuclear grade distinguishing these two groups. There were less ER-positive and PR-positive cases in pCR group than in incomplete response group, however, this was statistically significant only for PR. The pCR group showed significantly greater HER2 signals and HER2/CEN17 ratio than the incomplete response group. Nineteen cases showed HER2 intratumoral heterogeneity (30%) and significantly more cases with HER2 intratumoral heterogeneity were found in the incomplete response group (60%) than in the pCR group (10%).

		pCR		Incomplete response		p value
		# / average	% / range	# / average	% / range	
Total cases		39		25		
age		52	30-70	57	34-76	NS
Grade	Nottingham grade	2.56	2-3	2.48	1-3	NS
	Nuclear grade	2.85	2-3	2.8	2-3	NS
ER/PR	ER+	14	36%	17	68%	NS
	PR+	7	18%	13	52%	0.004
HER2 ISH	HER2 signals/cell	23.26	3.6-35.59	14.54	3.24-40.31	0.004
	HER2/CEN17 Ratio	8.32	2.28-29.98	5.08	1.23-14.99*	0.002
HER2 heterogeneity		4	10%	15	60%	0.00002

Table 1. Clinical and pathological results in all HER2-positive cases with neoadjuvant chemotherapy. Abbreviations: pCR: pathologic complete response; ISH: in-situ hybridization; CEN17: chromosome 17 centromere. *Note: Two cases in the incomplete response group had HER2/CEN17 ratio<2, but had HER2 signal >6.

Conclusions: HER2 intratumoral heterogeneity, as defined in this study, is associated with incomplete response to anti-HER2 targeted therapy in HER2-positive breast cancers.

213 Evaluating the Checkpoint Immune System in HER2-Positive Breast Carcinoma with Anti-PD-L1 Multiplex Immunohistochemistry and Its Association with the Response to Anti-HER2 Neoadjuvant Chemotherapy

Zaibo Li, Hiroaki Nitta, Peter Banks, Yanjun Hou, Anil Parwani. Ohio State University, Columbus, OH; Ventana Medical Systems, Inc, Tucson, AZ.

Background: Programmed cell death 1 (PD-1) together with its ligand (PD-L1) are key suppressors of cytotoxic immune response. The expression of PD-L1 in tumor cells and/or immune cells has been reported in breast cancer. However, its correlation with response to anti-HER2 neoadjuvant chemotherapy in HER2-positive breast cancer has not been reported. We evaluated the association between the checkpoint immune system with pathologic complete response (pCR) following anti-HER2 neoadjuvant chemotherapy in HER2-positive breast cancer.

Design: 64 HER2-positive cases of invasive breast carcinoma treated with anti-HER2 neoadjuvant chemotherapy were included. Multi-color multiplex immunohistochemistry with co-localization of PD-L1 with other immune markers (CD8 and CD163) was performed on whole sections from pretreatment biopsies and the following parameters were assessed, PD-L1-expressing tumor cells (PD-L1 TC), PD-L1-expressing peritumoral inflammatory cells (PD-L1 PTIC), PD-L1-expressing intratumoral inflammatory cells (PD-L1 ITIC), tumor infiltrating lymphocytes (TIL), peritumoral T cells (PTT), peritumoral macrophages (PTM) and tumor associated macrophages (TAM).

Results: PD-L1 expression was observed in tumor cells (9% of cases), TILs (17%) and peritumoral inflammatory cells (67%). PD-L1 expression was positively associated with high levels of TILs, PTTs and macrophages. 39 patients had pCR and 25 patients had incomplete response. There were significantly less PR-positive cases in pCR group than in incomplete response group. The pCR group showed significantly greater HER2 signals and HER2/CEN17 ratio than the incomplete response group. TILs and peritumoral lymphocytes/macrophages were positively correlated with pCR, and more importantly, PD-L1 expressing peritumoral inflammatory cells showed the most significant association with pCR.

		Complete response		Incomplete response		p value
		# or average	% or range	# or average	% or range	
Total cases		39		25		
Age		52	30-70	57	34-76	NS
Grade	Nottingham grade	2.56	2-3	2.48	1-3	NS
	Nuclear grade	2.85	2-3	2.8	2-3	NS
ER/PR	ER+	14	36%	17	68%	0.063
	PR+	7	18%	13	52%	0.004
HER2 FISH	HER2 signal	23.26	3.6-35.59	14.54	3.24-40.31	0.004
	Ratio	8.32	2.28-29.98	5.08	1.23-14.99*	0.002
Checkpoint immune system	PD-L1 TC	4	10%	2	8%	0.763
	PD-L1 PTIC	32	82%	11	44%	0.002
	PD-L1 ITIC	9	23%	2	8%	0.119
	PTM	37	95%	19	76%	0.026
	PTT	32	82%	13	52%	0.010
	TIL	12	31%	2	8%	0.032
	TAM	12	31%	2	8%	0.032

Table 1. Checkpoint immune system is associated with response to anti-HER2 neoadjuvant chemotherapy in HER2-positive breast carcinoma. Abbreviations: PD-L1-expressing tumor cells (PD-L1 TC), PD-L1-expressing peritumoral inflammatory cells (PD-L1 PTIC), PD-L1-expressing intratumoral inflammatory cells (PD-L1 ITIC), tumor infiltrating lymphocytes (TIL), peritumoral T cells (PTT), peritumoral macrophages (PTM) and tumor associated macrophages (TAM). *Note: Two cases in the incomplete response group had HER2/CEN17 ratio<2, but had HER2 signal >6.

Conclusions: The presence of PD-L1 expressing peritumoral inflammatory cells was the most significant positive predictor among checkpoint immune system factors for complete response to anti-HER2 targeted therapy in HER2-positive breast cancers.

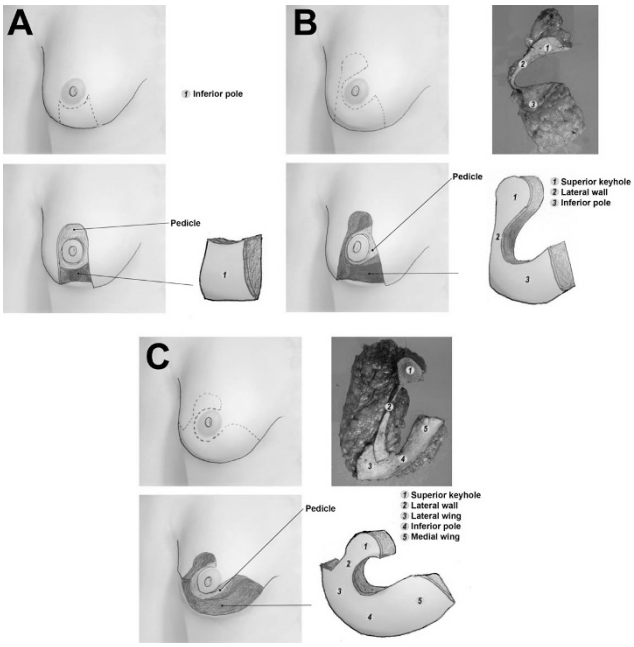
214 Anatomy and Terminology of Breast Oncoplastic Large Volume Displacement Surgery Specimens

Yuanxin Liang, Abhishek Chatterjee, Stephen Naber. Tufts Medical Center, Boston, MA.

Background: Oncoplastic surgery provides breast cancer patients aesthetic satisfaction without compromising disease-free survival or overall survival. Large volume displacement oncoplastic surgical techniques extend the option of breast conservation therapy. Dissection of these oncoplastic surgical specimens presents unique challenges for pathologists as they are more complicated compared to traditional excision or mastectomy specimens. The pathologist needs to correlate the surgical procedure with the anatomic terminology of specimens generated from large volume displacement oncoplastic surgical techniques.

Design: To illustrate the anatomy, we collaborated with our surgeons to photograph five common oncoplastic specimens and create anatomic drawings to illustrate the orientation of these specimens within the breast. These steps form the foundation for producing specimen diagrams documenting the location of sections taken for histologic examination.

Results: Specimens include a circumvertical skin incision pattern with a superior or superomedial pedicle (A,B), or an inverted T (Wise) skin pattern with an inferior pedicle (C).



Specimen configuration requires specific notation such as "superior margin, medial wing" when inking surfaces and identifying location of histologic sections in the specimen. Review of histologic slides is aided greatly by using a detailed specimen diagram showing the location of each section and, in multipart specimens, for showing the relation of the different parts to one another. Photographs of the specimen following inking of surfaces provides an important reference as well. Whenever possible, orienting the specimen with the surgeon is recommended.

Conclusions: Large volume oncoplastic breast specimens can be challenging to orient and identify the location of histologic sections. Use of standard oncoplastic specimen nomenclature in pathology reports assures the surgeon and the pathologist of the precise location of critical histopathologic findings. Specimen diagrams and photographs document locations of histologic sections and aid the pathologist in reporting key findings in oncoplastic breast specimens.

215 A Five-Plex IHC Assay for Detecting the Status of Hormone Receptor, Her2 and 'Basal-Like' Subtype on FFPE Tissues Demonstrates High Concordance to Single IHC Tests in Invasive Breast Cancer

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Background: Basal-like subtype accounts for ~15% of all breast cancers and is associated with poor prognosis. Gene Expression Profiling (GEP) technologies are not practical for routine clinical ancillary workup due to high cost and technical complexity; therefore IHC surrogate tests have been widely utilized. Nestin and CK5 were the top two positive basal markers among 46 proposed basal markers surveyed against a GEP gold standard.[1] In this study we have combined these two basal markers with ER, PR, and Her2 to develop a five-plex antibody-panel IBC classifier that provides hormone receptor (HR) and Her2 status, in addition, to identifying basal-like subtype.

Design: The five-plex IHC antibody cocktail was made by mixing well-established clones of ER, PR, HER2, CK5 and Nestin. The antibodies were detected with HRP or AP conjugated polymers. Rabbit monoclonal antibodies ER (SP1) and PR (YR85) were visualized together as brown nuclear staining and HER2 (SP3) as brown membranous staining, while mouse monoclonal antibodies CK5 (XM26) and Nestin (10C2) were visualized together as red cytoplasmic staining. The five-plex assay was optimized on known tissue controls and verified on three FFPE tissue microarrays containing duplicate cores of a total of 210 breast cancer cases. Each core was scored and the positivity of HR and Her2 staining was determined according to ASCO/CAP guidelines, and that of CK5 and nestin using a ≥5% cutoff. For the purposes of this study, concordance was defined as positivity resulting from the five-plex cocktail of a core to that of the same core resulting from a single stain.

Results: A total of 204 out of 210 invasive breast cancer (IBC) cases were analyzed after removing cases missing cores or with inadequate tumor cells. Among them, 190 cases were in complete concordance between the multiplex assay and all five individual IHC assays, yielding a complete concordance rate of 93.1%. The concordance rate for HR status alone was 97.5%, for Her2 status alone was 98.5%, and for basal markers CK5 and nestin combined positivity was 97.5%.

Conclusions: The five-plex IHC assay for examination of HR status, Her2 and 'basal-like' subtype on IBC FFPE tissue microarrays demonstrated high concordance with corresponding individual IHC tests. The assay is simple to use and cost effective compared to more complex technologies. The utility of this five-plex assay should be further validated as a surrogate for IBC classification.

216 Discrepancy Among Oncotype DX Testing, Immunohistochemistry, and Fluorescence *In-Situ* Hybridization Methods in Evaluating Breast Cancer Biomarker Status

Suzanna J Logan, Chao Zhang, Yuan Liu, Momin T Siddiqui, Cynthia Cohen, Xiaoxian Li. Emory University, Atlanta, GA.

Background: Oncotype DX (ODX) testing uses reverse transcriptase polymerase chain reaction (RT-PCR) to predict distant recurrence rate of estrogen receptor (ER)-positive, lymph node-negative breast cancers. ODX testing also reports the status of breast cancer biomarkers, including ER, progesterone receptor (PR), and HER2 status, using RT-PCR analysis. This study examined the discrepancy rate of breast cancer biomarker status as reported by ODX testing and routinely used immunohistochemistry (IHC), and fluorescence *in-situ* hybridization (FISH) methods.

Design: A total of 610 ER+ breast cancer cases with ODX reports were reviewed. ER, PR, and HER2 status from ODX reports were compared with results from IHC and/or FISH studies from the respective pathology reports.

Results: Twelve of the 610 cases (1.9%) were reported ER negative by ODX. Of these 12 cases, 4 (33.3%) showed weak ER immunopositivity (1-10%) and 8 (66.67%) demonstrated strong ER immunopositivity (>10.0%) by IHC examination. Of the 610 cases, 117 (19.1%) were reported PR negative by ODX. By contrast, IHC showed 19 (16.2%) of the 117 cases were weakly PR positive and 37 (31.6%) were strongly PR positive. Five of the 610 cases were reported as HER2 positive by FISH analysis; however four of these five cases (80.0%) were reported as HER2 negative by ODX testing. One recent unequivocal HER2 positive case by IHC (**Figure 1**) was reported HER2 negative by ODX testing (this case was not included in the original study of 610 cases).

Conclusions: The discrepancy rate of biomarker status between ODX testing and routine pathology reports using IHC and FISH methods is high.

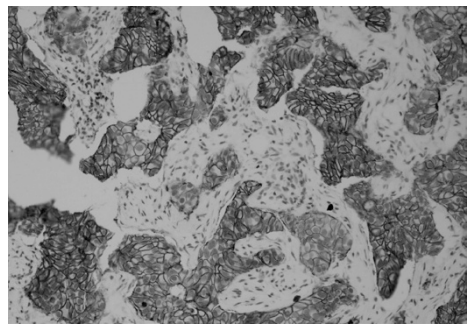

Figure 1. This case demonstrated strong, complete membrane staining of the tumor cells (3+). The same case was reported as HER2-negative by Oncotype DX testing. No FISH testing was performed. Magnification: 200x.

217 Estrogen Receptor-Positive/HER2-Negative and Lymph Node-Negative Breast Cancers Have Similar Oncotype DX Recurrence Score Distribution and Prognosis in African American and Caucasian Patients

Suzanna J Logan, Chao Zhang, Yuan Liu, Momin T Siddiqui, Cynthia Cohen, Xiaoxian Li. Emory University, Atlanta, GA.

Background: Oncotype DX (ODX) testing has been widely used to predict distant recurrence in estrogen receptor-positive (ER+)/HER2-negative (HER2-) and lymph node-negative (LN-) breast cancers. This study aims to examine the distribution and prognostic value of the ODX breast recurrence score in African American and Caucasian patients.

Design: A total of 604 ER+/HER2-/LN- patients with an ODX recurrence score from 2006 to 2014 were retrieved from our institution. Among these patients, 369 (61%) were Caucasian, 185 (31%) were African American, and 50 (8%) were either another race or missing race information. ODX score distribution, metastasis (other than lymph node metastasis), disease-free survival (DFS), and overall survival (OS) times were compared between African American and Caucasian patients, using the Chi-Square test or Cox proportional hazard model.

Results: There was no statistically-significant difference in ODX score distribution between African American and Caucasian patients (low-risk: 50.3% African American vs. 58.3% Caucasian; intermediate-risk: 35.7% African American vs. 32% Caucasian; and high-risk: 14.1% African American vs. 9.8% Caucasian; P = 0.41). No significant

difference between African American and Caucasian patients was observed in metastatic rate (hazard ratio [HR] = 1.94, P = 0.60), DFS time (HR = 0.84, P = 0.54), or OS time (HR = 3.59, P = 0.380).
Conclusions: African American and Caucasian patients with ER+/HER2-/LN- breast cancer have similar ODX recurrence score distribution and prognosis (*i.e.*, incidence of metastasis; DFS; and OS).

218 Lymph Node Metastasis Is Not Predictive of Overall Survival, Disease Free Survival, or Metastasis in Estrogen Receptor-Positive/Her2-Negative Breast Cancer

Suzanna J Logan, Ceyda Sonmez, Chao Zhang, Cameron Neely, Pia Mendoza, Cletus Arciero, Yuan Liu, Xiaoxian Li. Emory, Atlanta, GA; Georgia State, Atlanta, GA.
Background: Estrogen receptor-positive (ER+)/Her2-negative (HER2-) breast cancer has a good prognosis. The role of lymph node metastasis in ER+/Her2- breast cancer is not well defined. This study examined the association between lymph node (LN) status (positive vs. negative) and overall survival (OS), disease free survival (DFS), and distant metastases (DM), in ER+/Her2- breast cancer patients.
Design: A total of 603 ER+/Her2-/LN- and 94 ER+/Her2-/LN+ breast cancer patients diagnosed from 2006 to 2014 were identified. LN status (positive vs. negative), race, age at diagnosis, tumor size, Ki-67 score, ER and progesterone receptor (PR) status, lymphovascular invasion (LVI), Oncotype DX score (ODX), and Nottingham tumor grade were correlated with OS, DFS, and DM, by univariate and multivariate analyses.
Results: LN metastasis was significantly associated with presence of LVI, larger tumor size, and receiving chemotherapy (all p<0.001). In univariate analysis (Table 1), high-risk ODX, presence of LVI, and increased age at diagnosis were associated with worse OS (all p<0.05); LN metastasis, high-risk ODX, Nottingham tumor grade 3, presence of LVI, receiving chemotherapy were associated with worse DFS (all p<0.05), while younger age at diagnosis was associated with improved DFS (p<0.05); LN metastasis, high-risk ODX, negative PR expression, Nottingham tumor grade 3, presence of LVI, and receiving chemotherapy were associated with DM (all p<0.05). In multivariate analysis, only LVI was associated with worse OS (hazard ratio [HR]=5.96, 95% CI=2.05-17.35, p=0.001); and only receiving chemotherapy was associated with worse DFS and higher risk of distant metastasis (both p<0.01).
Conclusions: Presence of LVI, but not LN metastasis, correlates with worse OS of ER+/HER2- breast cancer. The relative importance of LVI and LN status should be confirmed in a larger study.

Univariate Analyses			
Outcome	Covariate	HR	P-value
OS	High ODX	4.63	0.012
	LVI	5.80	<0.001
	Older age at Dx	1.06	0.024
DFS	LN mets	2.30	0.010
	High ODX	2.64	0.022
	Nottingham grade 3	5.23	<0.001
	LVI	2.01	0.019
	Chemo	6.33	<0.001
	Younger age at Dx	0.97	0.028
DM	LN mets	4.71	<0.001
	High ODX	7.29	0.004
	PR negativity	3.93	0.016
	Nottingham grade 3	10.07	0.002
	LVI	3.58	0.006
	Chemo	9.52	<0.001

219 Improving Visual Quantification of Ki-67 Proliferative Index Using NDER, a Web-Based Training Platform

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Background: Immunohistochemistry for Ki-67, a marker of cell proliferation, is an important prognostic/predictive tool in breast carcinoma cases. The percentage of positive carcinoma nuclei (%Ki67) can be estimated by visual means. However, problems exist with inter/intra-observer variability, and software-based methods have been developed for objective quantification. However, these methods can be time-consuming, technically challenging, and are not available in all practice settings. Given the potential value of improving visual interpretation of %Ki67, we developed a module using a web application called Novel Diagnostic Electronic Resource (NDER), which uses rapid visual training to improve user %Ki67 quantification.
Design: Whole-slide images of breast carcinoma stained for Ki-67 were annotated with regions of interest, and images were extracted using custom software. The %Ki67 was quantified using the ImmunoRatio ImageJ plug-in, and the algorithm was adjusted to optimize scoring. The quantified images were categorized as follows: 0-5%, 5-15%, 15-30%, 30-50%, and >50% to create an approximately 20 minute, 167 image module using the NDER training platform. Training images are displayed briefly (average: 4 sec.), and users select an answer with immediate feedback. A pilot study of 11 pathologists and trainees was performed.
Results: Users improved in pre-to-post test accuracy (57% vs.73%, p<.005). The effect size (Cohen's d=1.35) was very large, where 0.2 is small, 0.5 moderate, and 0.8 large. Improvements were highest in the 0-5% (48.5% to 87.9%) and 30-50% categories (33.3% to 60.6%). Users did not improve in the 5-15% category (66.7% to 66.7%) and improved modestly in the 15-30% category (42.4% to 51.5%), which had relatively few cases. Accuracy dramatically rose in cases of lobular carcinoma (36% pre to 73% post). Per user observations, the software appeared to count non-neoplastic nuclei in some cases, falsely lowering %Ki67.
Conclusions: Substantial improvement in visual quantification of %Ki67 was achieved using the NDER application. The concordance between visual and software-based quantification was poor, and despite marked improvement, only reached 73%. Limitations include nonspecific counting by the computer software, highlighting the need for visual %Ki67 assessment in certain situations. The dramatic user improvements in this pilot study suggest a need for further investigation of training tools like NDER to maximize the reproducibility of visual Ki67 assessment relative to software-based quantification.

220 Massively Parallel Sequencing Analysis of Myxoid Fibroadenomas Reveals a Genomic Landscape Distinct from That of Conventional Fibroadenomas

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Background: Myxoid fibroadenomas (MFAs) are characterized by a distinctively hypocellular myxoid stroma. MFAs can occur sporadically, or in the context of the Carney Complex, an inheritable autosomal dominant condition due to inactivating mutations of the *PRKAR1A* gene. Conventional fibroadenomas (FAs) harbor highly recurrent mutations affecting exon 2 of the *MED12* gene. We sought to define the genomic landscape of MFAs and compare their repertoire of genetic alterations to that of conventional FAs.
Design: DNA samples extracted from 11 MFAs and matching normal tissues were subjected to massively parallel sequencing (MPS) using an assay targeting all exons of 410 cancer-associated genes and non-coding and regulatory regions of selected genes. Somatic mutations, insertions/ deletions and copy number alterations were detected using state-of-the-art bioinformatics algorithms. Mutation rates affecting single genes in MFAs were compared to those in previously reported conventional FAs.
Results: MPS yielded a median coverage of 560x (269x-745x), identifying a median of one (range 0-3) somatic mutation per MFA. No MFA harbored *PRKAR1A* germline mutations or occurred in a patient known to have Carney Complex. No recurrent mutations were identified in MFAs, with each case displaying a unique repertoire of somatic genetic alterations and a low mutation burden. One MFA displayed a somatic loss-of-function mutation in *PRKAR1A;* upon histologic re-review, this case was reclassified as a breast myxoma. Single cases harbored a hotspot mutation in *TP53* (R248W, not coupled with loss of heterozygosity), a hotspot *PIK3CA* mutation (H1047L) and a disruptive deletion in *PIK3R1* (L347del). One case displayed multiple gains and losses, and a focal amplification affecting *FLT4*. No MFA harbored *MED12* mutations, which were found to be significantly less frequent in MFAs than in conventional FAs (0 vs 72%, p<0.001, Fisher's exact test).
Conclusions: MFAs are genetically heterogeneous and differ from conventional FAs by a lack of *MED12* mutations. A somatic inactivating *PRKAR1A* mutation was identified in a breast myxoma, which is consistent with the spectrum of tumors observed in Carney Complex patients.

221 Clinicopathologic Characteristics and Survival Outcomes of Small Size Breast Carcinoma with Metastasis

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Background: The size of the primary tumor in breast cancer is one of the most important prognostic factors. A linear correlation between the size of the primary tumor and the presence of lymph node metastases and clinical outcome has been shown in the literature. However, data on small tumor size (pTmi, pT1a, pT1b) with local or distant metastasis at presentation is limited. Our aim was to study the clinicopathologic characteristics of this cohort of early breast cancer.
Design: After institutional review board approval, all the cases recorded between 1990-2013 in the cancer registry of the health system for breast cancer were included in this study. Cases with tumor size ≤1 cm (pTmi, pT1a, pT1b) were identified. Clinicopathologic characteristics including age at diagnosis, histologic subtypes, grade, receptor status, TNM stage and outcomes were noted. Statistical analysis was performed Fisher's exact test (two-tailed P-value <0.05 significant) and Chi square test (P-value <0.05 significant).
Results: Total of 7512 cases of invasive breast cancers were identified. 77/889 (8.7%) pTmi, T1a, pT1b patients with metastasis at presentation with no prior history of ipsilateral / contralateral malignancy or neoadjuvant therapy. There were 47 (61%) Caucasian (CA), 28 (36%) African American (AA), 2 (3%) Others. Mean age was 60yrs (30-87yrs). Average follow-up was 87 months (16-216 months) with 3, 5 &10 yr. year survival rates of 97% (69/71) and 93% (54/58) and 68% (9/28) respectively. The pathologic characteristics were:18/77 (23%) Grade1, 32/77 (42%) grade2, 24/77 (29%) grade3, 5 cases (6%) didn't have information on grade. Hormonal status: 48/77(62%) ER+Her2-, 12/77 (16%) ER-Her2-, 8/77 (11%) ER-Her2+, 2/77(3%) ER+Her2+, & 6/77 (8%) Her-2 Status unknown.

Table 1- Clinical parameters		
Clinical parameters	Sub-categories	Number of Patient (%)
Race	Caucasian	47/77 (61%)
	African American	28/77 (36%)
	Others	2/77 (3%)
Clinical stage grouping	IA	4/77 (5%)
	IIA	65/77 (84%)
	IIIA	3/77 (4)
	IIIC	5/77 (7%)
Survival rate	3 years survival rate	69/71(97%)
	5 years survival rate	54/58 (93%)
	10 years survival rate	19/28 (68%)
Table 2: Pathologic characteristics		
Pathologic characteristics	Sub-categories	Number of Patient (%)
pT stage	pTmi	3/77 (4%)
	pT1a	11/72 (15%)
	pT1b	63/77 (81%)
pN stage	pT0(i+)	4/77 (5%)
	pNmi	28/77 (36%)
	pN1a	37/77 (48%)
	pN2a	3/77 (4%)
	pN3a	5/77 (7%)
pM stage	pM0	77/77 (100%)
Grade	G1	18/77 (23%)
	G2	32/77 (42%)
	G3	22/77 (29%)
	Grade not determined	5/77 (6%)
Histopathologic subtypes	IDC-micropapilary type	5/77 (7%)
	IDC with Paget's disease	2/77 (3%)
	IDC NOS	58/77 (75%)
	IDC & ILC (mixed type)	2/77(3%)
	ILC	8/77 (10%)
	Medullary carcinoma	1/77 (1%)
	Mucinous carcinoma	1/77 (1%)
ER status	ER +	55/77 (71%)
	ER -	22/77 (29%)
Her-2 status	Her-2 +	8/77 (10%)
	Her 2 - (ER+/Her-2-)	48/77 (62%)
	Her 2 - (ER-/Her-2-)	12/77 (16%)
	Her-2 equivocal	3/77 (4%)
	Her-2 status unknown	6/77 (8%)

Conclusions: Compared to SEER CSR breast cancer data, small size tumor with lymph node metastasis at the time of diagnosis didn't predict more unfavorable prognosis in our cohort. None of the cases had distant metastasis in this cohort. There were more CA patients but no age difference compared to the SEER population. Although there was no significant difference noted in histologic subtype distribution, invasive ductal carcinoma micropapillary subtype appeared to be at a higher frequency in the small size tumor with LN metastasis.

222 Clinical Impact of Second Opinion on Lesions of the Breast and Axillary Region

Vicente Marco, Felip Garcia Hernandez, Isabel T Rubio. Hospital Quironsalud Barcelona, Barcelona, Spain.

Background: Histopathological assessment provides the foundations to select the appropriate treatment of breast tumors. A second opinion may be requested when a patient is transferred to another hospital for treatment. The aims of this study were to analyze the impact of *major discrepancies* on treatment of lesions of the breast and axillary region.

Design: 304 patients referred from other institutions to the Breast Oncology Unit were selected for pathology review, after clinical evaluation. Cases were classified as *major discrepancies*, after pathology second opinion, when it was considered that the differences had the potential to significantly impact in prognosis and treatment. The differences in therapeutic recommendations were analyzed.

Results: *Major discrepancies* were detected in 56 patients (18.42%). They were related to histological classification (27 cases), including benign versus malignant, breast cancer versus non-breast cancer, histologic type of breast cancer, and grade; the presence or absence of invasion, microinvasion, and ductal carcinoma in situ (15 cases), and the results of biomarkers predictive of response, hormone receptors and HER2 (17 cases). As a result of major discrepancies, 23 patients avoided systemic breast cancer treatment (chemotherapy, Herceptin, or radiotherapy). In 4 patients diagnosis of breast cancer changed to lung cancer or vice versa, with the benefits of a different chemotherapy. In 2 cases each, chemotherapy, Herceptin, and radiotherapy were indicated. In 1 patient mastectomy was avoided. But in 2 patients unnecessary mastectomy had already been performed for tumors of the axilla thought to be metastases from occult breast cancer, prior to second opinion, which were reclassified as metastatic melanoma and primary axillary adnexal tumor, respectively. In 14 cases *major discrepancies* had no significant clinical impact.

Conclusions: Second opinion in the pathology of breast and axillary lesions has the potential for detection of *major discrepancies*, that significantly impact in the selection of appropriate treatments. Ideally all the cases should be reviewed, however, selection based on clinical evaluation is useful. The magnitude of clinical impact is multifactorial, including the time of detection of *major discrepancies* and type of diagnosis involved.

223 Basaloid Salivary Gland-Like Carcinomas of the Breast (BSGLC). Histopathology and Prognosis

Vicente Marco, Felip Garcia, Isabel T Rubio, Teresa Soler, Laura Ferrazza, Ignasi Roig, Inmaculada Mendez, Xavier Andreu. Hospital Quironsalud Barcelona, Barcelona, Spain; Hospital Universitari Bellvitge/ICO/IDIBELL, Hospitalet de Llobregat, Spain; Consorci Sanitari de Terrassa, Terrassa, Spain; Hospital General de Granollers, Granollers, Spain; Hospital Parc Tauli, Sabadell, Spain.

Background: BSGLC are rare tumors with histologic features overlapping with adenoid cystic carcinoma (ACC) and other triple negative, basaloid breast carcinomas. The reported experience on their diagnostic criteria, and biologic behaviour is very limited. The aim of this study is to present clinical, histological and immunohistochemical features of BSGLC, and report on their prognosis.

Design: Eleven cases were collected from the participating institutions after reviewing cases coded as ACC and basaloid carcinoma. Hematoxylin and eosin stained slides and paraffin blocks were available in all cases. Immunohistochemical stains (IHC) with antibodies reactive with p63, c-kit, Myb, estrogen/progesterone receptors, and Her2 were performed. MYB FISH was studied in 10 cases. Follow-up was obtained in ten cases.

Results: The mean age was 56.6 years (range 44 to 75). The mean tumor size was 23mm (range, 15 to 45 mm). The patients were treated with breast conserving surgery and sentinel node biopsy (n 9) and mastectomy (n 2). Six patients received chemotherapy. One patient presented a sentinel node micrometastasis. Ten patients were alive without evidence of disease at a mean follow-up of 68.4 months (range 10 to 228 months), and one case was lost to follow-up. The main histologic features were solid pattern will cellular nests showing basaloid rimming. The tumors were well circumscribed, and the stroma showed dense collagen. Two tumors showed focal squamous differentiation. Ten tumors were high grade. Five tumors were p63 positive, 11 were c-kit positive, and 3 were Myb positive. MYB FISH was positive in 1 of 10 cases studied. All tumors were triple negative (ER-/PR-/HER2-).

Conclusions: BSLGLC of the breast are triple negative tumors. They may show overlapping features with solid variant and high grade ACC, but they lack the characteristic pseudocysts and cribriform pattern of ACC. They seldom involve lymph nodes. This series suggests that they have limited tendency to lymph node metastases, and good prognosis.

224 Flat Epithelial Atypia (FEA) in Directional Vacuum-Assisted Biopsy (DVAB) of Breast Microcalcifications: Surgical Excision Is Not Necessary

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Background: The aim of this study was to analyze clinicopathological features of patients with FEA diagnosed on DVAB targeting microcalcifications to identify the upgrade rate to in situ ductal or invasive breast carcinoma (DCIS or IBC), and to determine factors predicting the presence of carcinoma in the subsequent excision.

Design: We retrospectively evaluated the histological, clinical, and mammographic features of 71 cases from 67 women with DVAB-diagnosed FEA with or without architectural atypia/atypical ductal hyperplasia (ADH) who underwent subsequent segmental excision. Patients with ipsilateral DCIS, IBC, or a mass lesion were excluded. The extent and percentage of microcalcifications sampled by DVAB was evaluated by mammography. All biopsy slides were reviewed and the following data were recorded: number of TDLUs involved, nuclear atypia, presence of associated necrosis, and ADH. ADH was further quantified as <5% of FEA, ≥5% to ≤2 TDLUs and > 2TDLUs. The segmental excision findings regarding presence of DCIS or IBC were recorded.

Results: The histologic findings from the DVAB and subsequent excision are summarized in the following table.

	Pure FEA (%)	FEA+<5%ADH	FEA+≥5%≤2TDLUs ADH	FEA+≥5%>2TDLUs ADH
N (%)	19 (26.7)	31 (43.6)	12 (16.9)	9 (12.8)
FEA involving ≤ 2 TDLUs	4 (5.6)	2 (2.8)	3 (4.1) *	0
FEA involving > 2 TDLUs	15 (21.1)	29 (40.8)	9 (12.8)	9 (12.8)
* 1 case of tubular caricnoma on excision				

Extent of calcifications ranged from <1cm (n=40), 1-3 cm (n=16), to >3cm (n=11). Only one case of tubular carcinoma (TC) (0.16 cm) was found in the biopsy site on excision, (upgrade rate of 1.4%). In this case, the FEA was associated with ADH in ≥5% to ≤ 2TDLUs with 50-90% of calcifications removed by DVAB and it was not seen on mammographic examination.

Conclusions: Our study adds to the growing evidence that diagnosis of FEA on DVAB for microcalcifications as the only imaging finding is not associated with a significant upgrade to carcinoma on excision, and therefore, excisional biopsy is not required. Additionally, excision may not be necessary for FEA with ADH limited to less than 2 TDLUs, provided that at least 90% of the calcifications have been removed on DVAB.

225 "Quality, Not Quantity": 10X Hot-Spot (HS) Analysis of Lymphocyte Markers (CD3, CD8, CD4, CD20) in Tumor-Infiltrating Lymphocytes (TILs) Is Superior to Whole Tumor (WT) Analysis in Triple-Negative Breast Cancer (TNBC)

Patrick J McIntire, Paula Ginter, Lina Irshaid, Xiaoyan Cui, Zhengming Chen, Sandra J Shin. Weill Cornell Medicine, New York, NY.

Background: TILs have emerged as a prognostic indicator of disease-free survival (DFS) and overall survival (OS) in TNBC. Defined as all mononuclear cells within the tumor, TILs are mostly composed of CD3+ cells with the majority co-expressing CD8+ and less CD4+. Many studies have shown a positive effect of CD8+ TILs, however, the data regarding CD4+ TILs are conflicting. Few studies have assessed CD20+ TILs. Surprisingly, the majority of these published studies were performed on tissue microarrays, a method which is least likely to be representative of TILs. In this study, we aimed to evaluate an alternative approach to assessing lymphocyte markers in TILs compared to the gold standard of whole tumor (WT) analysis using imaging analysis software.

Design: Immunohistochemistry (IHC) for CD3, CD4, CD8, and CD20 was performed, each on one representative whole tissue section from 76 cases of primary TNBC. Imaging and quantification of WT and 2.2 mm diameter (equivalent to one 10X field) of highest immunoreactivity (HS) for stromal and intratumoral CD3+, CD4+, CD8+, and CD20+ TILs were performed using HALO™ imaging analysis software (Indica Labs; Corrales, NM). Statistical analyses were performed using DFS and OS (range: 16 to 196 months, mean: 110 months) as primary endpoints and results from the HS versus WT (gold standard).

Results: CD3+ and CD8+ TILs were significantly correlated with DFS using either WT (CD3: *P*=.0221, CD8: *P*=.0114) or a 10X HS field (CD3: *P*=.0063, CD8: *P*=.0058). In addition, CD4+ TILs was significantly correlated with DFS using a 10x HS field (*P*=.0231) For OS, evaluating a 10X HS field for CD3+ and CD8+ TILs was found to be statistically significant (CD3: *P*=.0400, CD8: *P*=.0381) while none was found in any of the markers studied when using WT. CD4+TILs were only observed to be significantly associated with DFS using a 10X HS field (*P*=.0231). CD20 did not correlate with outcome using either method.

Conclusions: In theory, enumeration of immunoreactive cells by image analysis on WT should represent the gold standard in assessing TILs, however, we found that analyzing a single, smaller (10X) field found to have the highest concentration of immunoreactive cells (HS) to be a better predictor of long-term clinical outcome of TNBC. Our findings support the use of HS evaluation in the assessment of immunoreactivity of TILs.

226 Clinicopathologic and Immunophenotypic Characterization of Angiomatosis of the Breast: A Rare Vascular Entity Mistaken for Low-Grade Angiosarcoma

Patrick J McIntire, Paula Ginter, Sandra J Shin. Weill Cornell Medicine, New York, NY.

Background: Angiomatosis of the breast is a rare, benign vascular lesion often mistaken for low-grade angiosarcoma (LGAS), particularly in core biopsy material. While the histopathologic features of this entity have been described in occasional published reports, it has not been well characterized by immunohistochemistry including proliferation (Ki-67) index. We sought to better characterize this entity, particularly in finding distinguishing features from LGAS.

Design: Cases of mammary angiomatosis were identified in our breast pathology consultation files spanning 16 years (2000-2015). All available clinical and pathological material for each case were reviewed. Immunohistochemistry for CD31, D2-40 and Ki-67 was performed on a representative whole tissue section from each case.

Results: Eight cases from seven patients were identified for study. For one patient, both the primary and recurrent tumors were evaluated. All patients were female with a mean age of 48 years (range: 19-63 years). All were unilateral (left: 5/8, right: 3/8). Most presented with a palpable abnormality or mass (5/8) while fewer were detected by imaging (3/8). The mean tumor size was 4.1 cm (range: 2-9 cm). All cases showed variable sized ectatic, thin walled vessels lined by flat normochromic endothelium diffusely infiltrating stroma. Where present, lesional vessels infiltrated between terminal duct lobular units (TDLUs) but not into the intralobular stroma of TDLUs. Most cases (6/8) showed a combination of lymphatic-appearing and hemangiomatous appearing vessels, the latter notably lined by thin muscular walls. Of the remaining 2 cases, one case showed only lymphatic-appearing vessels and the other showed only hemangiomatous -appearing vessels. CD31 was diffusely positive in all cases. Lymphatic-appearing vessels were D2-40 positive in all but one case. D2-40 was negative or weak in 5/8 hemangiomatous-appearing vessels. Ki-67 indices were <1% in all but one case (5%). Overt features of malignancy including endothelial cell nucleoli, endothelial tufting, papillary formations, solid/spindle cell foci, blood lakes, mitoses, and necrosis were absent in all cases.

Conclusions: Mammary angiomatosis is a rare vascular lesion which typically presents as a palpable mass. Despite its characteristic diffuse and infiltrative growth, angiomatosis does not invade into intralobular stroma, an important distinction from LGAS. They are immunoreactive for CD31 and variably so for D2-40. A low (<1%) Ki-67 index helps to distinguish angiomatosis from LGAS which typically shows a higher (>20%) index.

227 Tissue Microarray (TMA) Based Immunohistochemical Studies of Lymphocyte-Specific Markers Yield Inaccurate Results

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Background: Tumor-infiltrating lymphocytes (TILs), in particular cytotoxic (CD8+) T-cells, have been associated with increased survival in patients with triple-negative breast cancers (TNBC). A surprising majority of immunohistochemical studies of TILs have been performed using a TMA, typically consisting of 0.6 to 1.5 mm diameter tissue cores. While not explicitly stated, it is unlikely that these TMAs were made for the purposes of studying lymphocyte specific markers and, thus, inaccurately capturing the extent of TILs given their heterogenous distribution near tumor cells. The purpose of this study was to identify the smallest area of analysis necessary to accurately assess a lymphocyte specific immunohistochemical marker while maintaining a prognostic value based on the hypothesis that a TMA core is too small for accurate analysis of this kind.

Design: IHC for CD8 was performed on a TMA slide containing 76 cases of primary TNBC and the corresponding whole tissue slides. Imaging and quantification of combined stromal and intratumoral CD8+ T-cells was performed using HALO™ imaging analysis software (Indica Labs; Corrales, NM) on the following sized areas of analysis: 0.6 mm diameter TMA core, 1.1 mm diameter (equivalent to 20X field), 2.2 mm diameter (equivalent to 10X field), 6 mm diameter field and whole tumor. Evaluation of the 1.1 mm diameter, 2.2 mm diameter, and 6 mm diameter areas was performed in the same region which was visually determined to have the highest proportion of CD8+ TILs ("hot-spot"). Statistical analyses were performed using disease-free survival (DFS) (range: 16 to 196 months, mean: 110 months) as a primary endpoint of 76 TNBC cases.

Results: In all sized areas of analysis performed on the whole tissue section (1.1 mm, 2.2 mm, 6 mm and whole tumor), increased CD8+ TILs were significantly correlated with DFS (*P* = .0033, .0058, .0111, and .0114, respectively), and additionally, the 2.2 mm (10X) "hot-spot" field was significantly correlated with OS (*P* = 0.0381). CD8+ TILs analyzed on the TMA core were not found to be significantly correlated with DFS (*P* < .05).

Conclusions: Using HALO™ Image analysis software, we have objectively quantified CD8+ TILs, a marker shown to have predictive and prognostic significance in TNBC, in graduated areas of tumor. We conclude that immunohistochemical analysis of lymphocyte specific markers in TMA yields inaccurate (underestimated) results which raises concern of the validity of published results using this platform in this investigative setting.

228 Low Progesterone Receptor Expression Is Associated with Distant Metastasis in Estrogen Receptor Positive/HER2 Negative and Lymph Node Negative Breast Cancer

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Background: Estrogen receptor positive (ER+)/HER2- and lymph node negative (LN-) breast cancers generally have good prognosis. Some have low progesterone receptor (PR) expression. The role of PR in these breast cancers is not well studied.

Design: A total of 604 ER+/HER2-/LN- breast cancer cases with Oncotype DX (ODX) recurrent scores diagnosed from 2006-2014 were retrieved from the archives of our institution. Correlation of PR expression with race (black vs white), ODX score, tumor stage, Nottingham tumor grade (1/2 vs 3), lymphovascular invasion (LVI), chemotherapy and radiation therapy, overall survival (OS), disease free survival (DFS) and metastasis was examined through Cox proportional hazard model.

Results: In univariate analysis, low PR expression (as continuous variation) was significantly associated with higher ODX score, lower ER expression, Nottingham tumor grade 3, lower Ki-67 score and receiving chemotherapy (all P<.05). Comparing with breast cancers with strong PR expression (>10%), cancers with weak PR expression (1-10%) had significantly worse DFS (hazard ratio: 2.74 (95%CI:1.25-6.02), P=0.032); breast cancers with negative PR expression were associated with a higher risk of metastasis (hazard ratio: 5.41 (95%CI:1.45-20.18), P=0.012). In multivariate analysis, weak PR expression was still significantly associated with higher risk of metastasis (hazard ratio: 3.80 (95%CI:1.67-8.69), p=0.002) after adjusting for other covariates.

Conclusions: Breast cancer with low PR expression is associated with metastasis and other adverse prognostic factors. ER+/PR low breast cancers need further studies for optimal treatments.

229 Pathological Prognostic Factors in Estrogen Receptor Positive/Her2 Negative and Lymph Node Negative Breast Cancers

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Background: Estrogen receptor positive (ER+)/Her2- breast cancers without lymph node metastasis (LN-) generally have good prognosis. However, a small portion of the patients have distant metastasis and poor prognosis. This study is to evaluate prognostic factors in this specific group of breast cancers.

Design: A total of 604 ER+/HER2-/LN-breast cancer cases diagnosed from 2006-2014 were retrieved from our institution. Patient age, race, Oncotype DX (ODX) score, ER, progesterone receptor (PR), Ki-67 score, Nottingham tumor grade, lymphovascular invasion (LVI), tumor size and stage were correlated with overall survival (OS), disease free survival (DFS) and metastasis (distant metastasis other than lymph node metastasis) using Cox proportional hazard model.

Results: Univariate analysis showed: 1) higher ODX score, older age at diagnosis, receiving radiation therapy and presence of LVI were significantly associated with worse OS; 2) higher ODX score, weak PR expression, Nottingham tumor grade 3, presence of LVI, receiving chemotherapy and younger age at diagnosis were significantly associated with worse DFS; 3) higher ODX score, negative PR expression, Nottingham grade 3, presence of LVI and receiving chemotherapy were significantly associated with higher risk of metastasis (all P<0.05). Multivariate analysis showed: 1) LVI (hazard ratio (HR): 4.41 (1.21-16.05), P=0.025) and older age at diagnosis (HR: 1.07 (1.01-1.13), P=0.019) were significantly associated with worse OS; 2) Nottingham grade 3 (HR: 2.26 (1.08-4.71), P=0.03) and receiving chemotherapy (HR: 5.76 (2.91-11.40), P<0.001was significantly associated with worse DFS; 3) receiving chemotherapy was the only parameter significantly associated with metastasis (HR: 23.42 (2.95-186.22), P=0.003).

Conclusions: LVI and Nottingham tumor grade are important pathological prognostic factors in ER+/Her2-/LN- breast cancers.

230 Correlating Infrared Imaging Based Automated Breast Tumor Staging Models with Immunohistochemical (IHC) Stained Images

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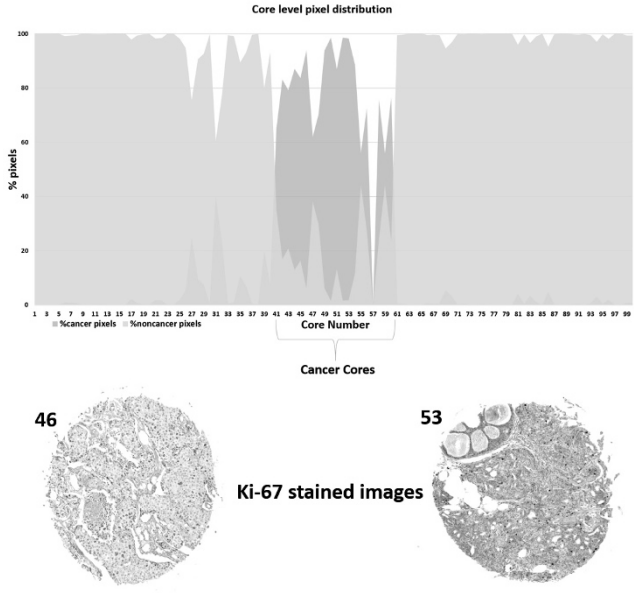
Background: Early breast cancer detection can greatly improve the patient outcome as the survival rates and prognosis is highly dependent on the stage of the tumor. Breast tissue is extremely heterogeneous making it difficult to understand tumor progression and the disease outcome. This study reports the comparisons and correlation of chemistry-based, high throughput approach utilizing classification models with IHC stained images especially the Ki67 stain. This would open new avenues for building semi-automated approaches complementing clinical diagnosis.

Design: Materials: Breast tissue microarrays (TMAs) obtained from US Biomax, Inc., were used for high definition (HD) infrared (IR) imaging. Hematoxylin and Eosin (H&E) and immunohistochemistry images were obtained using digital light microscopy. Corresponding 5 µm thick and 1mm in diameter sections were placed on a salt plate and imaged using infrared light.

Data Analytics: All the data was processed in ENVI + IDL using our in house programs. IR images were manually marked by mapping the annotated H&E stain---ed images under the supervision of a pathologist. The model differentiating malignant epithelial cells from benign is used to select different threshold ranges of the percentage of cancer pixels on each paitent biopsy. This information is then correlated to the epithelial staging model and sample proliferation status captured by the Ki67 stained image.

Results: The figure below shows the distribution of cancer pixels on each of the patient biopsy analyzed by an automated disease classification model. To the right of the pixel distribution plot, two patient samples stained with Ki-67 are shown which exhibit high proliferation. It is evident that the extent of proliferation captured by the stained image is also manifested as increased percentage of cancer cells detected by the model.



Conclusions: This study demonstrates the potential of HD IR spectroscopic imaging coupled to IHC staining, with inherent chemical information, to provide automated histopathology and cancer diagnosis in a quantitative and objective manner.

231 Pilot Study to Assess the Utility of Testing for Circulating HER2/Neu in the Serum of Breast Cancer Patients

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Background: Shedding of the extracellular domain (ECD) of HER2/Neu receptor is controlled by a metalloproteinase. Serum HER2/Neu ECD (sHER2) can be detected by ELISA kit by Nuclea Diagnostic Laboratories LLC, Cambridge MA, USA in breast cancer (BC) patients (pt). The aim of the study is to correlate sHER2 with tissue HER2 (tHER2; detected by IHC or FISH) and assess the implications of change in its levels during the course of disease.

Design: Serum samples were prospectively collected from 45 consenting pt. sHER2 levels were plotted on a standard curve and HER2 concentration calculated using a second order polynomial algorithm. Positive controls with various sHER2 levels were within the range specified by the manufacturer. The cutoff for positivity was 15ng/ml. Pertinent pathological and clinical data were analyzed.

Results: 73 sHER2 samples were obtained from 13 early BC, 17 locally advanced BC (LABC) pt and 15 metastatic disease (MD); in 28 pt (9, 10, 9 respectively), samples were obtained both pre and post therapy. 16 pt were tHER2+. sHER2 was positive in 6 pt (5 with MD, one with LABC); in 4 of them sHER2 was concordant with tHER2 (one was sHER2+/tHER-, the other was sHER2-/tHER2- at diagnosis but became sHER2+ when clinically progressed; table1). In 9 pt with early BC, 18 pre and post-surgery samples were sHER2- including one tHER2+ case.

Conclusions: The results of this pilot study confirm the technical validity, feasibility and patient's acceptance. Discordance between sHER2 and tHER2 may represent tumoral heterogeneity or extensive liver metastases affecting the degradation of HER2 ECD. In 3 patients increased sHER2 levels appear to correlate with disease progression. In LABC pt sHER2- post treatment was associated with pCR. sHER2 levels may potentially be used to monitor disease progression and predict response to therapy. A larger study is planned to confirm the clinical utility of this test in BC.

	Diagnosis/ grade	pT	LVI	LN	Metastasis	Response	IHC			Serum HER2 level (ng/ml)	
							HER2 IHC score	ER%	PR%	1st sample	2nd sample
METASTATIC	IDC/3	T3	N	Nx	L, B	*	3+	10	0	**46.4**	**62.9**
	IDC/3	T3	N	N+	L, B, skin, Br	*	1	90	-	10.9	**16.8**
	IDC/2	T3	N	N+	L, B, ovary	*	1	98	65	**16.6**	**16.8**
	Recurrent BC	Tx	U	N1	Lu, L, Br	*	3+	0	0	**70.6**	**77.4**
	IDC/3	T2	N	N1	L	pPR	3+	90	60	**33.9**	N/A
LABC	IDC/2 (inflammatory type)	T4d	N	N1	-	pCR	3+	95	0	**32.7**	5.9

N: Negative, N+: radiologically positive, U: unknown, *progression on several therapy lines, L: liver, Lu: lung, B: bone, Br: brain.

232 Comparing the Gene Xpert Breast Cancer RUO mRNA Assay with ER and HER2 Immunohistochemistry (IHC) for Rapid Biomarker Analysis

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Background: Care of patients with breast cancer in the developing world is limited by access to quality ER and HER2 IHC diagnostic assays needed to justify therapeutics. Shipping pathology specimens to a central testing site delays therapy and incurs high costs. The GeneXpert Breast Cancer STRAT4 (Research Use Only/RUO) assay makes qualitative measurements of ESR1, PGR, ERBB2, and MKi67 mRNAs from FFPE specimens in ~75 minutes on an automated RT-qPCR diagnostic platform, the GeneXpert (GX). Over 11,000 GX machines are in use in 182 countries, offering the possibility of near patient testing. We compared concordance between IHC and mRNA in breast tumors processed in Rwanda, with pathology review, GX, and IHC performed in the US.

Design: Both mRNA and standard ER and HER2 IHC assays were performed on 150 breast cancer FFPE samples with assays tested as whole sections. For IHC 2+, a positive FISH result scored the sample as HER2+. GX measurements for Ki67 were compared with mitotic rate as an alternative to Ki67 IHC.

Results: Valid IHC and STRAT4 results were available on 146 cases (90 cores, 56 excisions). Overall percent agreement was 93% for ER (51% +) and 97% for HER2 (27%+). 9/10 ER discrepancies were IHC ER+ and STRAT4 negative with 7/9 showing weak ER antibody staining (range 5-90%). 70% of ER discrepancies were in cores, of which 56% had small volume tumors ≤25mm^2 (34% overall) and 89% ≤50mm^2 (63% overall). 28/29 ER IHC- cases with a positive internal control (29/72) were appropriately identified as ER- by STRAT4. In 8/9 HER2 IHC2+ cases, STRAT4 correlated with FISH results, and excluding the IHC2+ cases, 2/3 discrepant HER2 were 1+ by IHC and STRAT4+; 1/3 was IHC+ and STRAT4-. Special subtype (lobular, mucinous, micropapillary) and low tumor cellularity (<50%) did not impact discordance. Comparing mitotic rate with MKi67 mRNA expression (8.2 mitoses/mm^2 cut point) gave 100% sensitivity and NPV, but low specificity (13.9%), and PPV (48%).

Conclusions: In this evaluation of samples processed locally in Rwanda, concordance was good for ER and excellent for HER2. Low tumor volume and/or weak expression of ER accounted for most discrepancies. The GX Breast Cancer STRAT4 Assay provides an on-demand solution to the problem of obtaining accurate diagnostic results in low resource settings.

233 Isolated Atypical Lobular Hyperplasia Diagnosed on Breast Biopsy: Low Upgrade Rate with Long-Term Follow-Up

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Background: Upgrade rates to cancer on surgical excision for atypical lobular hyperplasia (ALH) diagnosed on breast biopsy is controversial. Consequently, the need for surgical excision and the management of these patients is not well-established. We aimed to review cases with isolated ALH on biopsy to establish the rate of upgrade on excision and correlate with long-term follow-up.

Design: A database search was performed for a period of 188 months (1/00-8/16) to identify breast biopsies with a diagnosis of isolated ALH. Cases with any other atypical lesion in the concurrent biopsy or discordant radiologic-pathologic findings were excluded. Clinical-pathologic information was extracted from pathology reports and electronic records. Invasive carcinoma (IC) and ductal carcinoma in situ (DCIS) were considered upgraded pathology on excision. Patients with ALH on biopsy without and with a history of, or concurrent diagnosis of, breast cancer (IC or DCIS) were compared.

Results: 120 breast biopsies from 119 patients (age 54.5 ± 9.3 yrs) had isolated ALH. 83 (69.7%) had no history of and 36 (30.3%) had a prior or concurrent history of breast cancer. Mammographic calcifications were the most common indication for biopsy, followed by mass and asymmetry (76%, 9%, and 6%, respectively). The most common additional findings on biopsy were fibrocystic changes and fibroadenoma (82% and 11%, respectively). 80 (78%) cases underwent surgical excision with an overall upgrade rate of 3.8%. History or concurrent diagnosis of breast cancer did not significantly influence upgrade rate (p=0.2).

Surgical Excision: Most Atypical Diagnosis (n=80)	n (%)
Fibrocystic changes	24 (30%)
Fibroadenoma	2 (2.5%)
Papilloma	2 (2.5%)
ALH	19 (23.7%)
LCIS	23 (28.7%)
FEA	2 (2.5%)
ADH	5 (6.3%)
DCIS	3 (3.8%)
IC	0 (0%)
LCIS, lobular carcinoma in situ; FEA, flat epithelial atypia; ADH, atypical ductal hyperplasia	

Follow-up information was available for 78 patients (mean 73.2 ± 60.9 mos, range 6-261 mos). In patients with known excision status and follow-up (n=69), excision did not significantly reduce the overall rate of future cancer in the ipsilateral breast (p=1.0). Overall, 13% had a later diagnosis of breast cancer (in either breast), with mean time from ALH to cancer of 67 mos.

Conclusions: With careful radiologic correlation, the overall upgrade rate for isolated ALH on biopsy is low. Furthermore, surgical excision of ALH does not reduce the overall risk of future breast cancer; thus a more conservative approach for the majority of patients may be appropriate.

234 Metastatic and Hematolymphoid Neoplasms Involving the Breast: 20 Year Experience at a Large Tertiary Center

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Background: Metastases to and involvement by hematolymphoid neoplasms is rare in the breast.

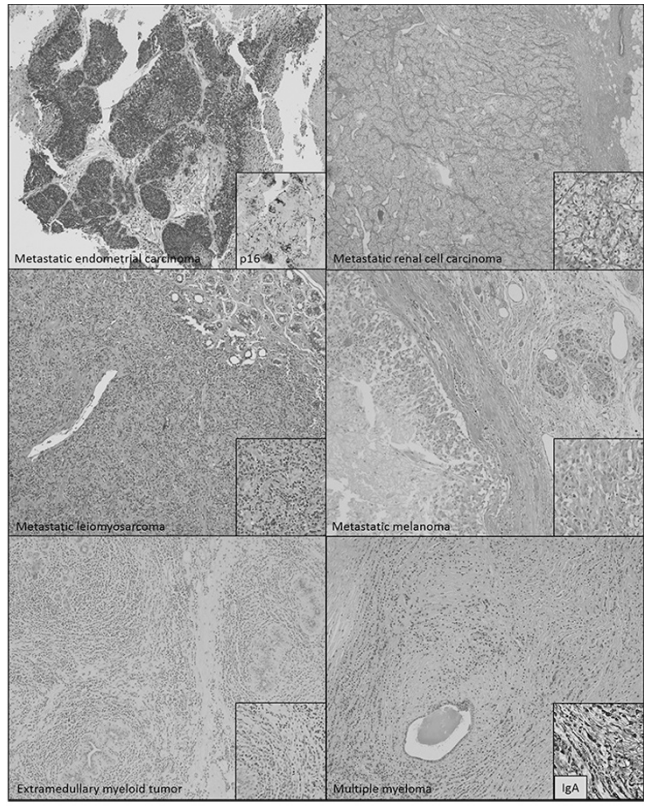
Design: Reports were reviewed following identification via electronic database search (1996-2016). Age; gender; general category of metastatic carcinoma, melanoma or sarcoma or hematolymphoid neoplasm; specific diagnosis; presentation and distribution (uni- or bi-lateral, solitary or multifocal/diffuse) in the breast and clinical outcome (ANED, LWD, DOD or DOC) were recorded.

Results: Of 36,991 breast pathology cases 72 (0.2%) had metastatic (carcinoma (17/72; 23.6%), sarcoma (10/72; 13.9%) and melanoma (10/72; 13.9%)) or hematolymphoid (35/72; 48.6%) involvement. The majority of patients were female (68/72; 94.4%) and mean age was 58 (range 25-93). 20 (27.8%) had initial presentation in the breast whereas 45 (62.5%) had known prior, pathology-confirmed tumor and 3 (4.2%) had no known prior diagnosis but clinical suspicion of a non-breast primary neoplasm (4/72; 5.5% unknown). Breast disease distribution was unilateral with single focus in 49 (68.1%), unilateral and multifocal in 8 (11.1%) and bilateral and multifocal or diffuse in 5 (6.9%) (10/72; 13.9% unknown).

Of 40 patients with follow-up information mean follow-up was 5.2 yrs and outcomes were: 9 (22.5%) ANED, 7 (17.5%) LWD, 23 (57.5%) DOD and 1 (2.5%) DOC. For those who DOD, mean time to death was 4.6 years (range 4 mos - 11.7 yrs).

Specific diagnoses are detailed in Table 1 and example cases are shown in Figure 1.

Lineage	Diagnosis	N (%)
Carcinoma (N=17)	Renal cell	6 (35.3)
	Lung	5 (29.4)
	Ovarian	5 (29.4)
	Endometrial	1 (5.9)
Sarcoma (N=10)	Leiomyosarcoma	8 (80)
	Liposarcoma	2 (20)
Melanoma (N=10)	Melanoma	10 (100)
Hematolymphoid (N=35)	Low-grade B-cell	23 (65.7)
	DLBCL	3 (8.6)
	T-cell	3 (8.6)
	Extramedullary myeloid tumor	3 (8.6)
	Burkitt	1 (2.8)
	Hodgkin	1 (2.8)
	Myeloma	1 (2.8)



Conclusions: Metastatic and hematolymphoid neoplasms rarely involve the breast. However, they most commonly (68.1%) presented as unilateral, unifocal disease, with breast involvement at initial presentation in 27.8%, potentially mimicking a primary breast neoplasm.

235 Clinico-Pathological Features of Breast Cancer Patients; Is It Different in an HIV Endemic Sub Saharan African Country- Botswana Experience

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Background: Breast cancer is the second most cancer among women in Botswana. Previous research from Western and Eastern Africa has shown that breast cancer tend to be more aggressive. Genetic diversity across the African continent makes it imperative to investigate whether this parallels differences in breast cancer biology. An effective anti-retroviral therapy programme for HIV has significantly increased the life span of people living with HIV (PLHIV) in Botswana. The prevalence and increased survival of PLHIV and the increasing incidence, prevalence of breast cancer have created a sizeable cohort of patients with this comorbidity. More information is needed about this emerging cohort in order to ensure optimal treatment. Aim of this study was to compare the pattern of hormone receptors expression with clinic-pathological parameters like patient's age, tumour size, type, grade, lymph node and HIV status.

Design: This study is a 5 year retrospective study using archival data from the National Health Laboratory in Gaborone from 01/1/2011 to 12/31/2015. Clinical records of patients were retrieved from the Integrated Patient Management System.

Results: 392 breast specimens analysed over a five year period from 1/1/2011 to 12/31/2015. The mean age of patients with breast cancer was 55.5. IHC data was available on 219 samples. Overall, 67.6% of tumours were ER positive, 51.1% of tumours were PR positive, and 9.1% were HER2+ positive. About one-fifth of tumours (20.1%) were triple negative. The most common (78.12%) subtype was invasive ductal carcinoma and grade (44.6%) was 2. Tumours ranged in size from <1cm to 16cm. The average tumour size found 4.3 cm. The largest prevalence of tumours were found in stage T2 (38.9%), while almost half the tumours staged had significant nodal involvement (stage N2a, 50%).

Conclusions: The prevalence of triple negative tumours in Botswana was higher than those documented in white populations (21% vs 10%-16%) but similar to that seen in black women in America (26%). The prevalence of triple negative cancer is similar to rates documented in other parts of sub Saharan Africa. The breast cancer in Botswana occurs in women of a younger age (54 vs 61) than the United States. Tumour characteristics in Botswana compared to the United States include: a lower prevalence of ER+ (67.6% vs 73.1% and similar HER2+ (9.2% vs 10%). HIV- individuals had a slightly higher prevalence of ER+ disease than HIV+ individuals (70.7% vs 82.9%). There was no difference in HER2 positivity among those who were HIV+ and HIV- (9.8% vs 7.3%).

236 Amyloid Precursor Protein as a Potential Marker of Malignancy and Prognosis of ER Negative Breast Cancer

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Background: Amyloid precursor protein (APP) is well known for its key player in Alzheimer's disease. Recently, its involvement in human malignancies begins to unravel. Nonetheless, its role in breast cancer has not been well addressed yet.

Design: APP expression in a cohort of invasive breast cancer was examined, and it was correlated with the clinic-pathologic features, biomarkers expression, molecular subtypes, and patients' survival. The functional role of APP expression was investigated in breast cancer cell line.

Results: APP expression was detected in 238/1131 cases (21%) of invasive breast cancer. It correlated positively with histologic grade, presence of necrosis, and lymphocytic infiltrates ($p<0.001$ for all), and negatively with age ($p<0.001$) and presence of fibrotic focus ($p=0.005$). There was no correlation between APP expression with tumor size, lymphovascular invasion, extensive intraductal carcinoma, tumor margin or lymph nodes status. For biomarkers expression, APP expression correlated negatively with ER and PR, and positively with Ki67, HER2 and basal makers ($p<0.001$ for all). For molecular subtypes, APP was least prevalent in luminal A cancers ($p<0.001$). In survival analysis, APP expression was associated with shorter disease free survival (Chi-square=10.599, $p=0.001$) and overall survival (Chi-square=12.648, $p<0.001$) in the entire cohort, and it was an independent unfavorable prognostic factor in ER negative cancers.

Consistently, in a panel of breast cancer cell lines, a lower APP expression was found in ER positive (MCF-7 and ZR75-1) than ER negative (SKBR-3, BT-549, MB-231 and MB468) cancer cell lines by western blotting analysis. The functional role of APP was further examined by siRNA knockdown in BT-549 cell line. siAPP knockdown BT-549 cells showed a significant reduction in the proliferation rate than those control siRNA transfected cells by WST-1 assay. They had also a reduced colony forming capacity than control cells. In transmigration assay, siAPP knockdown cells showed a marked reduction in migration through transwell compared to control.

Conclusions: APP expression was more prevalent in ER negative breast cancer and identified as an independent unfavorable prognostic factor in this subgroup of breast cancer. It appears to play multiple functional roles in breast cancer. Owing to its higher expression and function in ER negative cancer, it may be a useful therapeutic target in ER negative breast cancer.

237 Metabolic and Immune Features of High Risk DCIS

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Background: Ductal carcinoma in situ (DCIS) of the breast is a common diagnosis with the advent of radiological screening of large populations. The best treatment options for an individual patient with DCIS remains elusive, as there are no robust markers to identify women at high risk for developing invasive disease. Tumor metabolism and immune infiltrate are known to have impacts on cell invasiveness and metastatic progression, but the role in DCIS is largely unstudied.

Design: The association of markers of glycolytic metabolism (MCT4) and immunosuppressive macrophages (CD163) with risk of any recurrent ipsilateral breast event (IBE) or progression to invasive breast cancer (IBC) was analyzed using data from 236 DCIS patients treated with breast conserving surgery with long-term follow-up in the absence of radiation therapy. MCT4 and CD163 expression were assessed with immunohistochemistry using the respective validated antibodies (Santa Cruz, catalog#SC50329, dilution 1:200 and Cell Marque, catalog#163M-17, prediluted). The level of MCT4 staining was scored in DCIS tumor cells and in stromal compartments using previously published criteria. CD163 was dichotomized based on median expression. Hazard ratios (HR) were estimated using a univariate and multivariate Cox regression model and Pearson correlation coefficients were computed between all markers.

Results: High expression of MCT4 in DCIS tumor cells and in the stroma was strongly associated with risk of an IBE (HR=8.1, $p< 0.001$ and HR = 6.0, $p<0.001$ respectively) and IBC recurrence (HR=6.6, $p=0.0125$ and HR=18.8, $p=0.006$ respectively). The presence of CD163 positive cells was also strongly associated with risk of an IBE (HR=5.9, $p<0.001$) and IBC recurrence (HR=12.4, $p=0.016$). These markers remained statistically significant in multivariable analyses that included nuclear grade, necrosis, comedo subtype and ER and HER2. The glycolytic metabolic phenotype strongly correlated with the intensity of immunosuppressive macrophages infiltrate ($r = 0.60$, $p < 0.001$).

Conclusions: These studies indicate that expression of MCT4 and CD163+ macrophages defines a subtype of DCIS with high-risk for recurrence and progression. The absence of these markers delineates a form of DCIS with minimal risk of progression to invasive cancer, suggesting the possibility of using these markers for treatment decisions.

238 Comprehensive Genomic Profiling of Metastatic Malignant Phyllodes Tumors of the Breast

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Background: Metastatic malignant phyllodes tumors (MPT) are exceptionally rare and the genomic drivers of these tumors are being elucidated. We performed comprehensive genomic profiling of MPT to learn whether targeted therapies could be an option for patients with relapsed and refractory disease.

Design: DNA was extracted from 40 microns of FFPE specimen from 22 cases of relapsed, refractory and metastatic MPT. CGP was performed using a hybrid-capture, adaptor ligation based next generation sequencing assay to a mean coverage depth of 620X. Tumor mutational burden (TMB) was calculated from a minimum of 1.11 Mb of sequenced DNA as previously described and reported as mutations/Mb. The results were analyzed for all classes of genomic alterations (GA), including base substitutions, insertions and deletions (short variants; SV), fusions, and copy number changes including amplifications (amp) and homozygous deletions.

Results: The 22 women with relapsed and metastatic MPT featured a median age of 51 yrs (range 14-70 yrs). CGP was performed on the primary MPT in 14 cases and on metastasis biopsies in 8 cases. Median TMB for all MPT was 2.7 mut/Mb and no MPT had a TMB ≥10 mut/Mb. 18/22 evaluable tumors were all microsatellite stable (MSS). The most commonly mutated genes in this cohort were *TP53* (63.4%), *TERT* (55.6%), *NF1* (50.0%), *MED12* (40.1%), *CDKN2A/B* (31.8%), and *MLL2* (36.4%). 20/22 (90.9%) MPT harbored clinically relevant genomic alterations (CRGA) associated with therapies available on the market or under investigation in late stage clinical trials. Additional alterations in the PI3K/ATK/MTOR pathway were identified in 11/22 (50%) of samples. Targetable *KIAA1549-BRAF* or *FGFR3-TACC3* fusions were identified in 2/22 (9.0%) tumors.

Conclusions: More than 90% of MPT feature clinically relevant GA including the previously un-described high frequency of mutations of *NF1* whichrepresented by far the most common targetable GA in this study. 50% of MPT had alterations predicted to result in loss of *NF1* activity. *NF1* mutation does not significantly co-occur with mutations in any other gene or pathway commonly altered in MPT. Given the responsiveness of other solid tumors with *NF1* GA to MEK inhibitors such as trametinib and selumetinib, further investigation of targeted therapy for clinically aggressive MPT appears warranted.

239 Paucity of Atypical Epithelial Proliferations in 379 Pediatric Fibroepithelial Breast Lesions

Ugur Ozerdem, Fattaneh Tavassoli. Yale University School of Medicine, New Haven, CT.

Background: Fibroepithelial lesions are the most common breast tumor among adolescents and children. The frequency of atypical epithelial proliferation in this setting is not well established. Prior studies that included adult and pediatric patients have suggested a 2% prevalence of epithelial atypia involving fibroadenomas. Given the absence of data in current literature on epithelial atypia in pediatric fibroepithelial lesions, all pediatric fibroepithelial lesions in our files were reviewed retrospectively.

Design: Clinicopathological features of 379 mammary fibroepithelial lesions excised from patients, 10 to 17 years of age, during a 36 year period (1980 to 2016) were retrospectively reviewed. All but 2 cases were lumpectomies for mass lesions, while two cases represented incidental fibroadenomas encountered in reduction mammoplasties performed for symptomatic macromastia.

Results: The 379 patients ranged in age from 10 to 17 years with an average of 16 years. Youngest children had the lowest frequency of fibroepithelial lesions. A majority, 370 (97.8%), of the cases were fibroadenomas, while 7 cases (1.8%) were low grade (benign) phyllodes tumors and 2 cases (0.6%) were hamartomas. Five of the 379 fibroepithelial lesions (1.3%) showed ductal intraepithelial neoplasia 1b (atypical ductal hyperplasia) involving the lesion; these patients ranged in age from 14 to 17 years. Histologic examination revealed isolated ducts partially involved by atypical cells proliferating in a cribriform or micropapillary pattern spanning ≤2mm, morphologically similar to DIN1b (ADH) in adult women. Low risk ductal intraepithelial neoplasia (duct hyperplasia without atypia) was the most common form of ductal proliferation present in 32 cases (8.4%). Fourteen cases (3.6%) had overlapping morphologic features of fibroadenoma and tubular adenoma, five cases (1.3%) showed involvement by fibrocystic changes, 6 cases showed infarct (1.6%), and 5 cases (1.3%) had involvement of the fibroadenoma by pseudoangiomatous stromal hyperplasia (PASH).

Conclusions: Ductal intraepithelial neoplasia 1b (ADH) is rare among children, noted only in 1.3% of completely excised fibroepithelial lesions. However, this is only slightly less than the frequency reported for patients ranging in age from 10-72 years in a prior study (1.3 % versus 2 %, $P>0.05$, Chi-square with Yates' correction). Low risk ductal intraepithelial neoplasia (duct hyperplasia without atypia) remains the most common type of epithelial proliferation in pediatric fibroepithelial lesions.

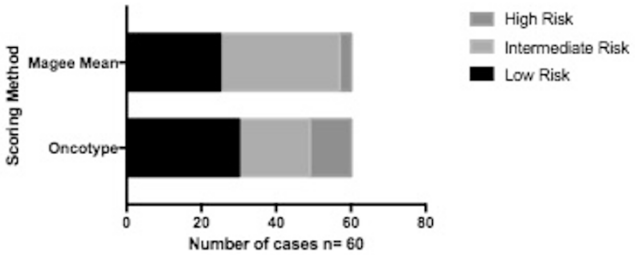
240 Comparison of Oncotype DX and Magee Recurrence Scores in 60 Infiltrating Breast Carcinomas

Ugur Ozerdem, Fattaneh Tavassoli. Yale University School of Medicine, New Haven, CT.

Background: Oncotype DX, a 21-gene assay that quantifies the10 year risk of distant recurrence, predicts benefit from chemotherapy in lymph node-negative, estrogen receptor (ER)-positive breast cancer. As a cost-effective alternative, the same tumors can be stratified into similar 3 risk groups using the freely available Magee Recurrence Score based on tumor grade, size, Ki67 index, ER H-score, PR H-score, and HER2 status. Only tumors with high risk scores benefit from chemotherapy. Current study compares the Oncotype DX recurrence score (OD-RS) with Magee recurrence scores (MRS) in 60 patients to elucidate the distribution of recurrence scores designated as low-risk (RS= 0-17), intermediate risk (RS=18-30), and high risk (RS= 31-100).

Design: MRS requires assessment based on 3 different equations depending on the type of information available for each tumor. The OD-RS and MRS (mean value of equations 1 to 3) were calculated on 60 ER-positive, lymph node-negative breast cancers for which Ki67 index, ER H-score, PR H-score, HER2 (IHC), HER2 (FISH), and grade were available. The results were compared using PRISM 7.0 statistical analysis software.

Results: OD-RS classified 30 cases as low risk, 19 cases as intermediate risk and 11 cases as high risk. The MRS classified 25 cases as low risk, 32 as intermediate risk, and only 3 as high risk.



Clearly, more cases qualified as intermediate risk based on MRS. This difference was statistically significant (P=0.0155, Chi square test). Bland-Altman plots displayed wider score differences between OD-RS and MRS among cases designated as high risk by OD-RS.

Conclusions: The Oncotype DX and mean Magee recurrence scores appear comparable in predicting "low risk" cases. However, substantially more cases fall in the "intermediate risk" category by MRS, while OD-RS qualifies more cases as "high risk." Modifications in existing MRS predictive equations may potentially result in a more comparable categorization. Either modification of MRS predictive equations or use of a two-tier system (high and low) with a different cut-off point may result in an optimal and more comparable categorization.

241 Oncotype DX Recurrence Score of Special Subtypes of Breast Carcinoma

Ugur Ozerdem, Fattaneh Tavassoli. Yale University School of Medicine, New Haven, CT.

Background: Oncotype DX is a commonly used commercial multi-gene assay for making chemotherapy decisions in ER positive, lymph node-negative breast cancers. Based on their recurrence scores (RS), such cancers are stratified into 3 categories: low risk = RS of 0-17, intermediate risk = RS of 18-30, and high risk = RS of 31-100. The RS of several special subtypes of breast carcinoma were assessed to determine if a consistent pattern would help in a more judicious approach to the use of this assay.

Design: In this investigation we compared the Oncotype-DX recurrence scores of mucinous carcinoma (n=22), cribriform carcinoma (n=3), tubular carcinoma (n=15), PR positive, pleomorphic invasive lobular carcinoma (PRP-PILC; n=20), PR negative, pleomorphic invasive lobular carcinoma (PRN-PILC; n=3), PR positive, classic invasive lobular carcinoma (PRP-CILC; n=15), PR negative, classic invasive lobular carcinoma (PRN-CILC; n=5), invasive carcinoma with osteoclast-like giant cells (n=2), and CK903-positive, E-cadherin-positive (hybrid positive/HP) tubulo-lobular carcinomas (HP-TLCA; n=5).

Results: The mean and median RS scores, respectively, were 17.6 and 16 for pure mucinous carcinoma, 10.3 and 11 for cribriform carcinoma, 12.9 and 14 for tubular carcinoma, 15.4 and 15 for hybrid positive tubulo-lobular carcinoma, 12.1 and 12 for PRP-CILC, 11.5 and 11.5 for invasive carcinoma with osteoclast-like giant cells, which fell into low risk group (RS=0-17). The mean and median RS for PRN-CILC, PRP-PILC, and PRN-PILC fell into the intermediate risk group (RS=18-30).

Conclusions: The mean and median RS for the 28 PR-negative and/or pleomorphic invasive lobular carcinomas were all in the intermediate risk category. On the other hand, PR positive, classic invasive lobular carcinoma, pure mucinous carcinoma, cribriform carcinoma, tubular carcinoma, hybrid-positive tubulo-lobular carcinoma, and invasive carcinoma with osteoclast-like giant cells all had a low mean and median RS. If these findings are confirmed in a larger group of cases, it could obviate performance of Oncotype DX assay at least on the morphologic variants of breast carcinoma that are known to have a more indolent behavior.

242 Estrogen Receptor-Positive and -Negative Adenomyoepitheliomas of the Breast Are Underpinned by Distinct Genetic Alterations

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Background: Breast adenomyoepitheliomas (AMEs) are uncommon lesions with dual epithelial and myoepithelial differentiation. AMEs are histologically heterogeneous, ranging from benign to high-grade tumors, which may be associated with carcinoma. Albeit reported to follow a benign clinical course, recurrences and metastasis can also occur. AMEs have been classically regarded as having a triple-negative phenotype, however estrogen receptor (ER)-positivity has also been reported. Here we aimed to determine the morphologic and genetic differences between ER-positive and ER-negative AMEs.

Design: 21 AMEs were subjected to whole-exome sequencing (n=6), targeted massively parallel sequencing (MSK-IMPACT, n=6) or Sanger sequencing analysis of *HRAS* and *PIK3CA* hotspot loci (n=9). The ER status of all AMEs was assessed by immunohistochemistry following current ASCO/CAP guidelines. Statistical comparisons were done using Fisher's exact test.

Results: 15/21 (71%) and 6/21 (29%) AMEs were ER-positive and ER-negative, respectively. ER-negative AMEs were associated with atypical morphologic features, such as stromal desmoplasia (p=0.03) and necrosis (p=0.02). 83% of ER-negative AMEs harbored *HRAS* Q61R or Q61K hotspot mutations, whereas *HRAS* Q61R mutations were identified in only 13% of ER-positive AMEs (p<0.01). The rate of hotspot *PIK3CA* mutations was numerically higher, though not statistically significant, in ER-negative AMEs (5/6, 83%) than in ER-positive AMEs (8/15, 53%; p>0.05). Concomitant *HRAS* and *PIK3CA* mutations, however, were significantly more frequent in ER-negative (67%) than in ER-positive AMEs (13%; p=0.03). Notably, the two AMEs with evidence of axillary lymph node dissemination were ER-negative and *HRAS*-mutant.

Conclusions: ER-positive and ER-negative AMEs differ histologically and genetically. ER-negative AMEs more frequently show atypical histology, are underpinned by recurrent *HRAS* hotspot mutations, and frequent concurrent *PIK3CA* mutations, alterations previously described in epithelial-myoepithelial carcinomas of the salivary glands. Our findings suggest that ER-negative AMEs could perhaps be considered the breast counterpart of epithelial-myoepithelial carcinomas.

243 Genomic Landscape of Phyllodes Tumors with and without Fibroadenoma-Like Areas

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Background: Breast fibroadenomas (FAs) and phyllodes tumors (PTs) are underpinned by recurrent *MED12* mutations, which are more frequent in FAs and benign PTs. In contrast, *TERT* genetic alterations are predominantly found in borderline and malignant PTs. There is evidence to suggest that a subset of PTs may arise from FAs, and have a better prognosis. We sought to investigate the genetic differences between malignant and borderline PTs with and without FA-like areas.

Design: We retrieved previously reported targeted massively parallel sequencing data on 19 PTs classified as borderline (n=6) or malignant (n=13) according to WHO criteria. Histological sections were reviewed to assess the presence of either intra- or pericanalicular FA-like areas, and the PTs categorized thereafter. Statistical comparisons were performed using Fisher's exact test and t-test.

Results: 12 PTs (4 borderline PTs and 8 malignant PTs) had FA-like areas, and 7 PTs (2 borderline PTs and 5 malignant PTs) did not. Medians of 3.5 (range 2-7) and 3 (range 2-8) non-synonymous somatic mutations were identified in PTs with and without FA-like areas, respectively (p>0.05). *MED12* mutations were found in 4/12 (33%) and 5/7 (71%) PTs with and without FA-like areas, respectively, whereas genetic alterations affecting *TERT* (*TERT* promoter mutations or *TERT* gene amplification) were found in 6/12 (50%) PTs with FA-like areas and 6/7 (86%) PT without FA-like areas. Mutations affecting *RARA* and *SETD2*, additional genes recurrently mutated in PTs, were found in 2/12 (17%) and 3/12 (25%) PTs with FA-like areas and 3/7 (42.9%) and 1/7 (14.3%) PTs without FA-like areas, respectively. 4/12 (33%) PTs with FA-like areas displayed mutations affecting *TP53* and *RB1*, each, as opposed to 2/7 (29%) PTs without FA-like areas, with mutations for each of those genes. *EGFR* was affected by mutations or amplification in 5/12 (42%) and 2/7 (29%) PTs with and without FA-like areas, respectively. No significant differences were identified in single gene comparisons between PT with and without FA-like areas (Fisher's exact tests, p>0.05).

Conclusions: Malignant and borderline PTs with FA-like areas do not seem to differ significantly at the genetic level from those lacking these areas. Additional analyses in larger cohorts are required to define potential differences between "*de novo*" PTs and those that may have stemmed from pre-existing FAs.

244 Pre-Operative Single-Fraction Radiotherapy for Breast Cancer Leads to Increased Tumor PD-L1 Expression by Transcriptome Sequencing

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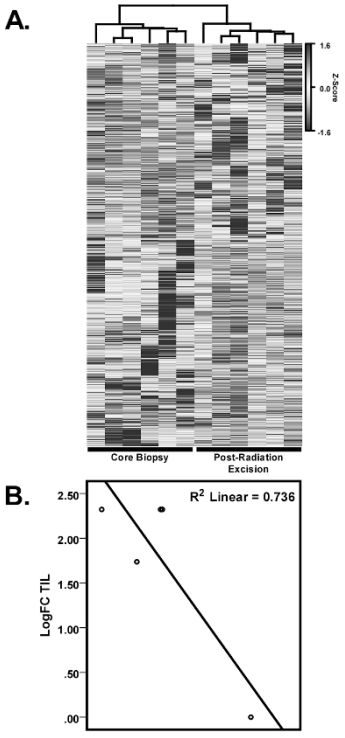
Background: Breast conservation surgery followed by radiotherapy has become the standard of care for early-stage breast cancer in the United States. However, *in vivo* tumor responses to radiation remain poorly understood and predictive markers in this context are lacking.

Design: RNA was extracted from paired core biopsy and excisional tumor tissue from a subset (n = 6) of patients from a larger ongoing trial consisting of women aged ≥55 years with node-negative, ER(+), HER2(-), T1 invasive carcinomas who received 21 Gy partial breast irradiation followed by lumpectomy within 10 days. RNA-seq was performed. Normalization and differential expression was carried-out using the EdgeR Bioconductor package and Gene Set Enrichment Analysis was used for pathway analysis. Tumor infiltrating lymphocytes (TIL) were assessed according to the International TILs Working Group 2014 recommendations.

Results: Pairwise analysis revealed differential expression in 10,840 coding genes (Fig. 1A), with significance-ranked enrichment for modulators of the immune response, including *CXCL2*, *AIF1*, *CXC4*, *IL8*, *MEFV*, and *FOS*, and mediators of the DNA damage response, including *CDKN1A* (p21). All cases demonstrated induction of PD-L1 (*CD274*)(LogFC=1.044; range 0.542–2.096; P<0.001), but not PD-L2 (*PDCD1LG2*) (P=0.094) or the PD-1 receptor (*PDCD1*)(P=0.094). Co-induction of the INFγ receptor (*IFNGR1*) was noted (LogFC=0.912; range 0.677–1.265; P<0.001). None of the cases demonstrated definite morphologic evidence for treatment effect. Plotting log fold change in PD-L1 expression against TILs yielded a coefficient of -0.736 (Fig. 1B).

Conclusions: Irradiation led to increased expression in mediators of the injury response. Although our series is small, there was consistent induction of the immune-suppressor PD-L1, and the degree of PD-L1 induction correlated with diminishing tumor infiltrating lymphocytes. With the emergence of immune checkpoint inhibitors as effective targeted therapy, these results suggest that testing for PD-L1 expression following radiation therapy may provide significant predictive information.



245 Concordance of Automated Image Analysis for *Human Epidermal Growth Factor Receptor2* (*HER2*) Fluorescent In-Situ Hybridization (FISH) with the Current Gold Standards in Clinical Practice

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Background: Accurate determination of *HER2* status is crucial to identify patients eligible for *HER2* targeted therapy. IHC and FISH are the gold standards for HER2 evaluation, however, they are fraught with subjectivity and interpretation errors. Sampling and tumor heterogeneity are the two major reasons for variability in assessment. In most institutions, FISH analyses are performed by cytotechnologists in a predetermined area of the tumour. These regions often have an admixture of invasive, in-situ and non-neoplastic processes that are difficult to differentiate on a fluorescent stained slide by untrained eyes. Image analysis platform enables the Pathologists to overlay the IHC, H&E and FISH scans to identify the region of interest. Our aim was to assess the performance of automated imaging for *HER2* FISH.

Design: The automated dual probe *HER2/CEP17* FISH processing was performed on Leica Bond III staining system at the Kingston General Hospital, Clinical Laboratory. The slides were scanned on Leica Ariol image analysis platform using z-stacking. The staining and scanning protocols were modified through several iterations of testing on over 100 'test' cases to achieve optimal signal strength. A subspecialty trained Breast Pathologist (SV) monitored and worked closely with the Leica team to optimize the parameters. Random cases were then evaluated with our Director for Cytogenetics for quality assurance. Finally, 31 new cases were used for step 1 of the validation. HER2 IHC followed by in situ hybridization on equivocal cases was performed as per the CAP guidelines. The scanned images were scored by a pathologist who was blinded to the results of prior HER2 analysis.

Results: Of the 31 cases, 29 had signal and background intensity optimal for scoring. 2 cases that were deemed suboptimal were rechecked on the Cytogenetics platform and were found to have artifacts due to poor tissue quality. 29/ 29 cases (100%) showed concordance between the automated HER2 staining and image analysis versus IHC/ manual FISH. The time taken for each assessment by the Pathologist was <10 minutes. Time consumed and ease of analysis was acceptable for incorporation in the routine workflow.

Conclusions: Our study shows that automated FISH analysis can be at par with the manual gold standard. Additionally, it is feasible to be incorporated in the routine workflow of the Pathologists at a tertiary consult center for the region. This has also the potential to overcome sampling and tumour heterogeneity issues.

246 Evaluation of Sentinel Lymph Nodes (SLN) in Classic Invasive Lobular Carcinoma (cILC): Study of 560 Cases Indicates Need for CK Staining and Inapplicability of Size Criterion (i.e. </>0.2 mm) to Distinguish pN0i+ from pNmi for Precise Staging

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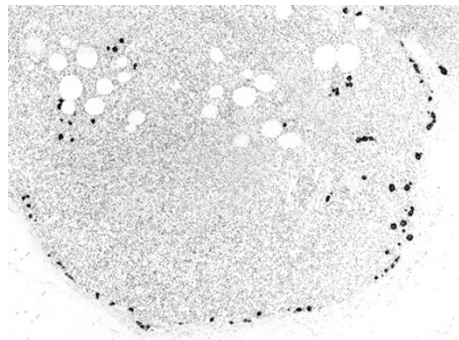
Background: Results of MIRROR trial (N Engl J Med 2009;361:653-663) emphasized need of additional therapy in cases of invasive breast carcinoma (ca) with isolated tumor cells (pN0i+) and micrometastasis (pN1mi) in SLN. However, assessment of SLN involvement in cILC is notoriously unreliable on H&E-stained sections alone (PLoS One 2014;9:e89778).

Design: Cases of cILC with H&E-/Cytokeratin AE1/AE3(CK)+ SLN, diagnosed over a 11.5 year (1/2005-6/2016) period, were reviewed. Only cases not readily identifiable on H&E were included. Pertinent clinical, pathological & follow-up data were obtained.

Results: 560 cILC cases with SLN were studied. 184/560 (33%) were SLN+, of which 43/560 (7.7%) SLN were H&E-/CK+. Following data relate to these 43 cases: mean age:61 (range:32-86); right:24 (56%), left:19 (44%); multifocal/multicentric cILC:22/43 (51%); mean size of cILC:2.0 cm (range:0.25-4.4); mean number of SLN:2.5; mean number of H&E-/CK+ SLN:1.4; cases with prior needle procedure or excision:43 (100%). CK+ cells were identified in isolation or in loose clusters, either in subcapsular sinus or nodal cortex or both.



CK+ cells did not form a distinct nodule (i.e. none was quantifiable in linear terms). 29 (67%) showed <200 CK+ cells (pN0i+) & 14 (33%) showed >200 CK+ cells (pN1mi). 16/43 (37%) underwent axillary lymph node (ALN) dissection, of which 5/43 (12%) were positive (>2 mm metastasis in ≥1 ALN). Ca recurred in 3/43 (7%). On statistical analyses, the number of CK+ cells (</>200) in SLN neither correlated with +ALN or with recurrences (due to small numbers of events in limited follow-up).

Conclusions: CK staining of SLN in cILC cases is imperative for precise staging. 7.7% of cILC showed H&E-/CK+ SLN. Distribution of CK+ cells precluded use of size criterion (</>0.2 mm) to distinguish pN0i+ from pN1mi; however, criterion of number of CK+ cells was readily applicable: 67% were pN0i+ (n:<200) & 33% were pN1mi (n:>200). Results of this study could be useful for cILC-specific revisions to the TNM Staging System.

247 Biomarker Testing Justification in Low Grade In-Situ and Invasive Breast Carcinoma – An Opportunity for Substantial Cost Containment: A Retrospective Analysis of 336 Cases

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Background: The College of American Pathologists/American Society of Clinical Oncology (CAP/ASCO) guidelines recommend estrogen receptor (ER), progesterone receptor (PR), and HER2 testing on all newly diagnosed invasive ductal (IDC) and ER testing of in-situ ductal carcinoma (DCIS). Recently this upfront biomarker testing of all breast cancers has been questioned, especially in DCIS. Biomarker testing also adds $1200 (CPT code 88360x3) and $400 charge to IDC and DCIS work-up, respectively. We sought to evaluate any pattern(s) of biomarker positivity in Nottingham histologic grade 1 (Gr1) IDC and low-grade DCIS. We assumed that Gr1 IDC is always ER positive and HER2 negative in order to determine the necessity of such testing, offer scientific proof to the medical community, and recommend avoiding such redundant practice to reduce unnecessary health care expense.

Design: A retrospective computer search, after IRB approval, retrieved DCIS and Gr1 IDC over a 6 years period. Patient age, tumor type, biomarker status, TNM pathologic stage, lymphovascular invasion, lymph node status, and nuclear grade were recorded.

Results: Clinico-pathological characteristics are summarized in Table 1 including Gr1 IDC, nuclear grade 1 (Nu1) and nuclear grade 2 (Nu2) DCIS.

Table 1: Clinico-pathological characteristics of patients with Gr1 IDC and low grade DCIS

	Gr1 IDC n=195	Low grade DCIS n=141
Age, years, average (range)	59 (32-91)	58 (30-86)
Size, cm, average (range)	0.3 (2.6-11.5)	3.0 (0.05-9)
ER Strong Moderate Weak Intensity NA Negative	100% 67% 15% 3% 15% 0%	100% Nu1, 95% Nu2 71% 11% 5% 9% 4%
PR Strong Moderate Weak Intensity NA	91% 44% 21% 12% 15%	N/A
Her2/neuScore 3 Score 2 Negative (score 0 and 1) NA	0.5% 33% 61.5% 5%	N/A
FISHNegative Equivocal	n=61 98% 2%	N/A

ER expression was observed in all Gr1 IDC (n=195), all Nu1 DCIS (n=24) and 95% Nu2 DCIS. ER expression was unrelated to presence of necrosis, calcification, and/or size.
Conclusions: Universal expression of ER in Gr1 IDC and in Nu1 DCIS is noted in this study. Therefore the testing for ER expression in Gr1 IDC and Nu1 DCIS is redundant and could safely be eliminated. The potential cost saving nation-wide would be huge. In this era of cost containment this should be judiciously looked into. Our findings seriously question the necessity of universal ER testing currently being practiced in USA.

248 Clinicopathological Characteristics of 815 Ductal Carcinoma In Situ (DCIS) from the BONBIS Trial – Focus on the HER2 Positive Group

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Background: Ductal carcinoma in situ (DCIS) is a heterogeneous disease in terms of clinical presentation, histopathological features, and underlying biology. For DCIS local treatment quality is a major prognostic factor as half of the recurrences are invasive. The phase III randomized trial "BONBIS" was designed to evaluate the impact of a 16-Gy boost after 50 Gy delivered to the whole breast in 25 fractions and 33 days in terms of local control. We present here the histopathological characterization of the 815 patients enrolled in BONBIS trial with a special focus of the HER2 positive DCIS.
Design: A total of 815 cases were available after the exclusion of 75 cases including 25 microinvasive DCIS. Central review of the histopathological features included grade (G), architecture, necrosis, abrasion, microcalcifications, inflammation, vascularization, mitotic index, ER, PR, HER2 and Ki67 immunohistochemical evaluation on slides. All the parameters were available for 788 pts. DCIS were classified according to an IHC-based intrinsic classification as luminal A: ER+ and PR+ and grade 1/2 / Ki67 low or and HER2-; Luminal B ER+ and PR- or HER2- or high grade/ high Ki67; HER2enriched: HER2 + and ER-; basal like: ER-, PR-, HER2-.
Results: G1: 16,4%, G2: 38,7%, G3: 44,9%; ER+81,3%; PR+:60%; HER2 positive: 26,2%; Ki67: 0-10%: 58,2%, 10-19%; 20,2%; >19% 21,6%; Luminal A:37,1%; luminal B: 44,3%; HER2 enriched: 10,9%; basal like:7,7%. In HER2 positive DCIS, the HER2 enriched were more frequently G3 than the luminal B HER2 positive (93% vs 65%, p<0,000028), of comedo architecture (41,90 vs 15% p=0,000015), less frequently cribriform (22,10 vs 40,80%; p=0,0048). HER2 expression was associated with inflammation and microvessels proliferation ($p<10^{-7}$).
Conclusions: This large prospective cohort of 815 centrally reviewed DCIS tumors from patients included in the BONBIS clinical trial of radiation therapy, shows a high proportion of HER2+ DCIS (26,2%), confirms a more aggressive features of this group and shows differences between ER+ and ER- HER2+ DCIS, as it is observed in invasive breast cancer. Data on the characterization of tumor infiltrating lymphocytes will be presented at the meeting.

249 Bilateral Prophylatic Mastectomy - A 5 Years Study from a Single Institution

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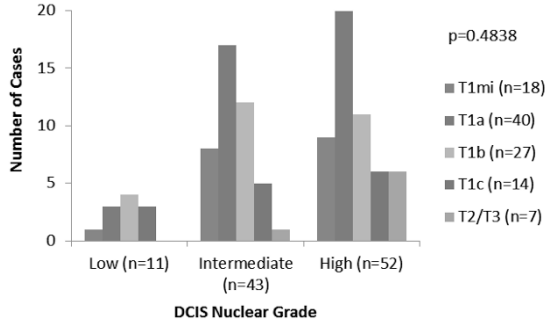
Background: Prophylactic mastectomy is a surgical procedure to reduce cancer risk in women with high predisposition. It can be done in 3 contexts, with different associated risks - patients without personal history of breast cancer with mutation in BRCA genes, with family history of breast cancer (bilateral prophylactic mastectomy - BPM) and patients with unilateral breast cancer (contralateral prophylactic mastectomy - CPM). Our aim is to characterize BPM, which provides a 90-95% cancer risk reduction in BRCA mutation carriers.
Design: We evaluated the BPM preformed at Instituto Português de Oncologia de Lisboa Francisco Gentil E.P.E. (IPOLFG), from 2009 to 2015. The clinical files were retrieved and the slides reviewed - in IPOLFG the BPM's parenchyma was entirely processed. Benign and malignant lesions were considered and the parenchyma's dominant pattern was classified as lipomatous or dense. We also reviewed the genetic test results.
Results: From 2009 to 2015, 20 women preformed BPM in IPOLFG. Ages ranged from 29 to 54 years and all were submitted to previous imaging (mammography and/ or ultrassonography), that classified 14 breasts as BIRADS 1, 25 as BIRADS 2 and 1 as BIRADS 4; in 1 case the imaging result was not available.
The breast parenchyma is predominantly lipomatous (55%), with a collagenous stroma (82,5%). The most prevalent benign lesions are sclerosing adenosis and ductal ectasia (57,5% each). We also found features of alterations of normal development and involution - ANDI (50%), cysts (35%), fibroadenomatoid hyperplasia (22,5%)/ fibroadenoma (20%), blunt duct adenosis (22,5%), apocrine adenosis (7,5%), common ductal hyperplasia (10%), 1 case of atypical hyperplasia, 2 papilomas and 1 hemangioma.
One invasive lobular carcinoma with 1mm was found (estimated cancer risk 5%).
50% of women have BRCA2 mutations, 20%, BRCA1 mutations, 15% didn't have BRCA mutations and 15% were not tested. The patient with invasive carcinoma had a BRCA2 mutation.
Conclusions: Our patients have a high percentage of BRCA2 mutations.
Processing the entire breast parenchyma we only found a 1mm invasive carcinoma, not visible by imaging, suggesting that total inclusion of BPM specimens has little value.

250 Feasibility of the Less Is More Approach in Treating Low Grade DCIS Diagnosed on Core Needle Biopsy: A 10 Year Review of DCIS Upgraded to Invasion at Surgery

Mirna B Podoll, Emily Reisenbichler, Andrew Bruner, Lania Roland, Sarah Mizuguchi, Mary Ann Sanders. Vanderbilt University, Nashville, TN; University of Louisville, Louisville, KY.

Background: Ductal carcinoma in situ (DCIS), commonly diagnosed by core needle biopsy (CNB), accounts for 20% of screen detected breast cancers. Reported rates of upgrade to invasive mammary carcinoma (IMC) at surgery range from 2% to 49%. Although DCIS is treated to prevent invasion, the likelihood that certain types, particularly low grade (LG) DCIS, will not progress to invasion suggests that we may be over treating these patients. Patients with DCIS have excellent survival and current trials are evaluating active surveillance versus conventional treatment for screen-detected LG DCIS. This study aimed to evaluate the feasibility of basing surgical treatment decisions on nuclear grade of DCIS on CNB.
Design: Pathology archives at two academic institutions were searched for cases of DCIS diagnosed on CNB with subsequent excision from 2006 to 2016. Clinicopathologic data was collected including nuclear grade of DCIS, mammographic findings, and combined histologic grade, receptor profile and stage of IMC on excision.
Results: We identified 1272 cases of DCIS on CNB: 201 (16%) were LG, 649 (51%) intermediate grade (IG), and 422 (33%) high grade (HG). Eleven (5.8%) of the LG, 43 (7.1%) IG, and 52 (14%) HG DCIS cases were upgraded to IMC on excision (Table). Overall upgrade rate was 8.3%. Of upgraded LG DCIS cases, all IMCs were ER positive/HER2 negative, histologic grade 1 or 2, and ≤pT1c (Figure). Six of the upgraded LG DCIS were associated with a mammographic mass. IG and HG DCIS cases accounted for all upgraded cases with HER2 positive IMCs (n=28), and all cases that were pT2/pT3 (n=7).

	Upgrade to Invasion		
	Yes(n=106)	No(n=1166)	p=0.0012
Nuclear grade			
Low	11	190	
Intermediate	43	606	
High	52	370	

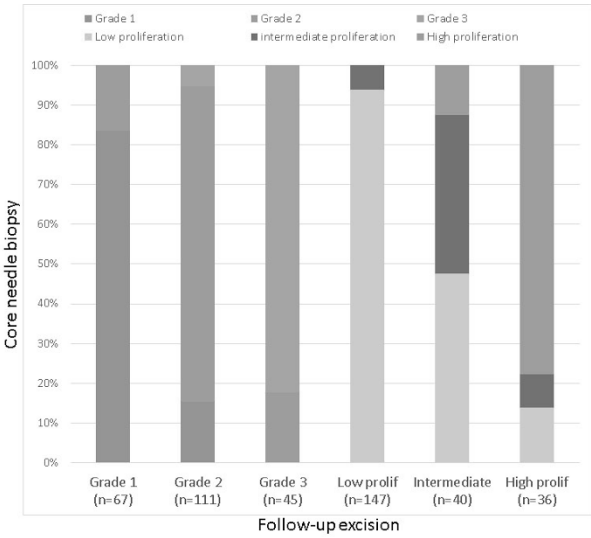


Conclusions: LG DCIS was significantly less likely to upgrade to IMC at surgery compared to IG and HG DCIS. All LG DCIS cases upgraded to IMC with favorable biology. Overall, omitting surgical treatment based on low nuclear grade alone may risk missing an upgrade to IMC. Including additional parameters, such as mammographic findings, could improve selection of patients with LG DCIS who could safely forgo surgery.

251 Correlation Between Invasive Mammary Carcinoma Grade in Ultrasound-Guided Core Needle Biopsy and Subsequent Surgical Excision

Mirna B Podoll, Melissa Straub, Stephanie N David, Mohamed M Desouki. Vanderbilt University Medical Center, Nashville, TN.

Background: The combined histologic grade of invasive mammary carcinoma (IMC) is a prognostic and predictive factor in identifying patients at high risk, and is one of the factors that determine therapeutic approach. The purpose of this study is to evaluate the correlation between the combined histologic grade of IMC and the mitotic rate, the least subjective component of grading, on ultrasound (US) guided core needle biopsy (CNB) and the subsequent follow up excision (FUE).
Design: A retrospective study of 223 consecutive IMCs were examined and biopsied under US guidance. Cases without subsequent excision and those with pre-excision neoadjuvant chemotherapy were excluded. Radiologic and pathologic data were retrieved from the patient's medical records. The modified Nottingham grading system was used. The mitotic rate was obtained by counting mitotic figures in 10HPF on the H&E slides.
Results: Needles of 14 and 12-gauge were used in 120 and 103 cases, respectively. The concordance, underestimation and overestimation of the grades between the CNBs and FUEs were 181 (81%), 25 (11%) and 17 (8%), respectively ($\kappa = 0.96$; 95% CI, 0.62-0.78; $r = 0.81$). CNB was less sensitive in identifying grade 2 (0.4) and more specific for grade 3 (0.97) tumors. Tumors >10 mm in size by US showed a greater concordance in the grade compared to ≤10 mm (83% vs. 77.0%, p=0.27). The overall concordance, underestimation and overestimation in the score of mitotic rate between the CNBs and the FUEs were 181 (82%), 27 (12%) and 13 (6%), respectively ($\kappa = 0.86$, CI, 0.51-0.72 and $r = 0.78$). Tumors ≤10 mm by US showed a greater concordance in the mitotic rate compared to those >10 mm (85% and 79%, respectively, p=0.23). Figure 1 summarizes grade and mitotic rate in FUE according to that in the CNB.



Conclusions: IMC tumor grade on CNB is important in triaging patients who may require systemic therapy prior to excision. US-guided CNB was found to predict FUE tumor grade and mitotic rate of IMCs in 82% of cases. Furthermore, CNBs were more specific in identifying high combined histologic grades with high mitotic rates. We propose that the limited material in CNBs, may explain the relatively high underestimation frequency of tumor grade and mitotic rate.

252 Ubc9 Expression in Correlation with BRCA1 in Hormone Positive (HP) and Triple Negative Breast Cancers

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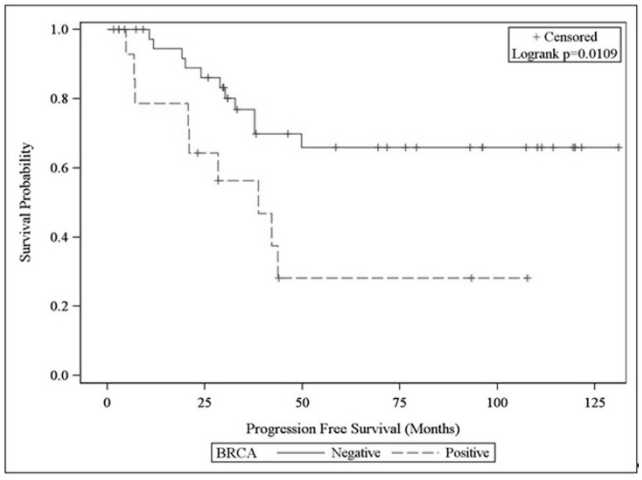
Background: Sumoylation is a post-translational modification linked to diverse cellular pathways, including cell growth, DNA damage response, proliferation and apoptosis. Ubc9, the sole Sumo-E2- conjugating enzyme, is a critical component of the Sumoylation machinery and has been linked to breast cancer pathogenesis, cell invasion, and metastasis independent of its ability to conjugate SUMO to protein substrates. BRCA1 binding to Ubc9 has been linked to ER transcription activity and triple negative breast cancer (TNBC). This study investigated Ubc9 and BRCA1 expression studied by immunohistochemistry (IHC) in hormone receptors (HR) positive, Her2 positive and TNBC tissue.

Design: Invasive mammary carcinomas (IMC) diagnosed during a 7-year period were reviewed. The IMC markers ER, PR, and Her-2 date were included. The tumors were studied as Her-2 positive, TNBC and HR positive. Tissue microarrays were stained with Ubc9 and BRCA1 monoclonal antibodies. The scoring of the IHC was semi-quantitative.

Results: Of the 166 cases studied, 93 were TNT, 65 were HR and 11 were Her2 positive. The age was 24-90 years of age. BRCA1 IHC was positve in 27.2% and Ubc9 in 51.3% of patients. We found statically significant positive correlation between BRCA1 and UBC9 expression in all carcinomas (Table 1).

			BRCA		
Covariate	Statistics	Level	Positive N=43	Negative N=115	P-value*
UBC9	N (Row %)	Positive	28 (36.36)	49 (63.64)	0.019
	N (Row %)	Negative	14 (19.18)	59 (80.82)	

BRCA-negative patients with non-TNT and with basal-like carcinoma had a better survival (p=0.0293 and p=0.0157). BRCA-negative patients with basal-like carcinoma had a better progression free survival (p=0.0109).



There were no statically differences in age, tumor size and lymph node metastases.

Conclusions: We describe novel expression patterns and association between UBC9 and BRCA1, Her2, and vimentin. This work supports a role for UBC9 protein expression, with or without BRCA in breast cancer pathogenesis.

253 Palpable Breast Masses in a Tertiary Institution in South-South Nigeria; Fine Needle Aspiration Cytology versus Histopathology: A Correlation of Diagnostic Accuracy

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Background: Fine needle aspiration cytology (FNAC) of the breast, as part of the triple assessment approach to the diagnosis of palpable breast lesions, has become a valuable pre-operative tool. It is fast, inexpensive and minimally invasive, and thus has gained wide acceptance in the pre-operative assessment of breast lesions. This study aims to determine the diagnostic accuracy of FNAC of palpable breast lesions in a tertiary hospital in Nigeria.

Design: This is a prospective comparative study comprised of the reports of the FNAC of palpable breast lesions and their subsequent tissue biopsy diagnoses recorded over a period of one year from 1st September 2013 to 30thAugust, 2014. Aspirates were obtained using 23G needle using either free hand or attached to 20 ml syringe/syringe holder. Smears were fixed in 90% alcohol and/or air dried and stained with Papanicolaou and Diff Quik stains respectively. Prepared slides were reported according to NCI guidelines. The initial cytological reports were correlated with the final histological diagnoses.

Results: A total of 100 consecutive FNAC reports were made during the study period. On cytodiagnosis; 14 (14%) cases were inadequate samples (C1), 52 (52%) cases were benign (C2) lesions, 2 (2%) cases were suspicious probably benign (C3) lesions, 4 (4%) cases were suspicious probably malignant (C4) lesions and 28 (28%) were malignant (C5) lesions. The cytology reports were correlated with the subsequent histological diagnoses. Of the 14 C1 reports, 13 were confirmed on tissue histology as benign lesions and the remaining 1 as malignant. 51of the 52 C2 reports were confirmed as truly benign (true negatives) and the remaining 1 as malignant (false negative). The overall suspicious rate (C3 and C4) was 6% with the 2 C2 reports confirmed as benign and 3 of the 4 C4 confirmed on histology as malignant. All 28 malignant (C5) reports were confirmed by tissue histology as malignant (truly positives). The absolute sensitivity was 84.9%, specificity was 98.2%, and positive predictive value (PPV) (C5) of 100%, complete sensitivity was 93.9%, Negative Predictive value (NPV) (C2) of 98.2%, false negative rate (FNR) and suspicious rate were 6.1% and 4.3% respectively.

Conclusions: FNAC of palpable breast lesions show high sensitivity and specificity in our centre. It is strongly recommended to be done on all patients presenting with palpable breast lesions to ensure quicker pathologist-surgeon communication, patients triage and early establishment of definitive treatment outline.

254 FOXA2 Protein Expression Is Associated with Recurrence in Patients with Triple-Negative Breast Cancer

Abdul Rehman, Hyunsung Kim, Yumin Chung, Youngchan Wi, Yeseul Kim, Su-Jin Shin, Seung S Paik, Kiseok Jang. Hanyang University, Seoul, Republic of Korea.

Background: The lack of available tailored targeted therapy due to immunophenotypic profiling of TNBC results in the poor clinical outcome. The forkhead box A2 (FOXA2), a transcription factor, has diverse functions ranging from embryogenesis to normal functioning of multiple adult tissues. Recent studies have demonstrated the tumor suppressor functions of FOXA2 in various human cancers; however, the role of FOXA2 protein in TNBC is not so well-defined. The objective of this study was to investigate the FOXA2 expression at the protein level in TNBC and to explore its relationship with various clinicopathological variables and prognosis of patients with TNBC.

Design: We examined FOXA2 expression at the protein level immunohistochemically in tissue microarrays consisting of 96 invasive TNBC cases from the institutional archives and interpreted the expression using a semiquantitative scoring system. For statistical analysis, immunoreactive scores of < 2 and ≥ 2 were considered low and high FOXA2 expression respectively. FOXA2 expression was correlated with various clinicopathological variables including patients prognosis. Chi-square test, Kaplan-Meier curves along log-rank test and Cox Proportional Hazard Model were used for statistical analyses.

Results: FOXA2 protein expression was detected in 43 (44.79%) of 96 TNBC tissues and 26 patients (27.08%) demonstrated low FOXA2 expression. The median age of patients was 51 years (range 26-79 years). The survival analysis revealed that TNBC patients with low FOXA2 expression had significantly shorter disease-free survival ($p = 0.040$, log-rank test) than those with high FOXA2 expression. Multivariate Cox regression analysis showed that lymph node metastasis was an unfavorable prognostic variable for recurrence in both disease-free ($p = 0.024$) and overall ($p = 0.002$) survival. However, no correlation between FOXA2 expression and clinicopathological parameters were observed.

Conclusions: Our results suggest that FOXA2 is significantly associated with relapse in TNBC and may function as a potential prognostic biomarker for patients with TNBC.

255 Overexpression of GATA4 Associates with Aggressive Phenotypes of Triple-Negative Breast Cancer

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Background: Triple-negative breast cancers (TNBC) represent a highly heterogeneous group of cancers that have the worst clinical outcome of all breast cancers. GATA4, a transcription factor, plays a pivotal role in the development of various organs, particularly cardiac development. Recent studies have demonstrated that GATA4 is expressed in several human malignancies, such as lung, ovary, breast, colon, pancreas, brain and stomach. However, little is known regarding the characteristics of GATA4 expression in TNBC. Therefore, we aimed to investigate the expression of GATA4 in TNBC and explore the relationship between its expression and major clinicopathologic variables including prognosis of patients with TNBC.

Design: We constructed tissue microarrays from formalin-fixed, paraffin embedded blocks of 93 invasive TNBC cases from an institutional archive. GATA4 expression was investigated immunohistochemically on tissue microarrays. The immunoreactive scoring system was employed to interpret GATA4 expression which was a product of staining intensity score (0-3) and proportion score (0-4). For statistical analysis, immunoreactive scores of < 2 and ≥ 2 were considered low and high GATA4 expression respectively. Association between GATA4 expression and various clinicopathological parameters including patients survival was analyzed. Chi-square test, Kaplan-Meier curves along log-rank test and Cox Proportional Hazard Model were utilized for statistical analyses.

Results: Nuclear GATA4 expression was detected in 44 (47.3%) TNBC tissue samples and 32 of 93 (34.4%) TNBC patients showed representatively high GATA4 expression. The median age of patients was 51 years (range 26-79 years). Overexpression of GATA4 protein was significantly associated with unfavorable clinicopathologic features, such as large primary tumor size ($p = 0.016$, χ^2-test), advanced AJCC stage ($p = 0.008$, χ^2-test), lymph node metastasis ($p = 0.016$, χ^2-test), lymphovascular invasion ($p = 0.016$, χ^2-test), and perinodal tumor extension ($p = 0.013$, χ^2-test). However, no correlation between GATA4 expression and survival outcome was observed in survival analysis.

Conclusions: Our results demonstrate that GATA4 is markedly expressed in TNBC and GATA4 overexpression significantly correlates with aggressive phenotypes of TNBC.

256 Potential Role of Desorption Electrospray Ionization (DESI) Mass Spectrometry Imaging (MSI) as an Adjunct to Histology for Margin Assessment in Lumpectomies for Breast Cancer

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Background: Obtaining negative margins in breast cancer surgeries is critical in preventing local recurrence. Re-operation rate due to positive margin is around 15% for lumpectomies, and could be improved by ancillary intraoperative tissue identification techniques. DESI MSI is an emerging technique capable of tissue characterization based on metabolite profiling. It can be applied to unstained frozen section slides to create tissue images based on the relative abundance of metabolites that topographically match the histology sections. We present a pilot study assessing DESI's ability to perform a simulated margin analysis in lumpectomies.

Design: Fresh tumor samples with adjacent non-neoplastic tissue were collected from 10 lumpectomies with biopsy-proven invasive ductal carcinomas. Each tissue edge of the specimen was defined as a simulated margin. Unstained frozen section slides were subjected to DESI MSI, and subsequently stained by hematoxylin and eosin (H&E) for histologic correlation.

Results: Metabolites with distinct mass-to-charge ratios (*m/z*) were found to be characteristically abundant in tumor (*m/z=863*) versus non-neoplastic tissue (*m/z=215*), respectively.

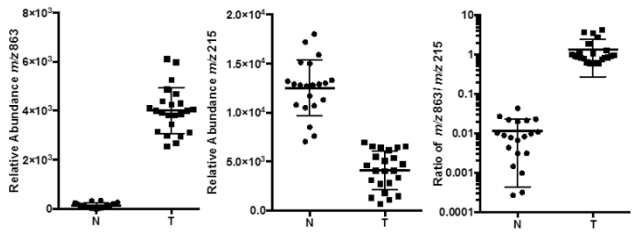

Fig 1. Dot plot showing characteristic separation of non-neoplastic (N) and breast carcinoma (T) tissue by the relative abundance of metabolites m/z 863 (A), m/z 215 (B), and the normalized ratio of m/z 863 against m/z 215 (C) from DESI data generated from histologically designated 20 non-neoplastic points and 20 breast carcinoma points in a representative breast cancer specimen with clearly defined tumor and non-neoplastic junction.

DESI MSI based on individual metabolite was able to accurately highlight tumor at tissue margins in samples with large tumor nests. MSI based on the ratio of *m/z=863* and m/z=*215* further improved DESI's ability to detect infiltrative smaller tumor clusters at tissue margins.

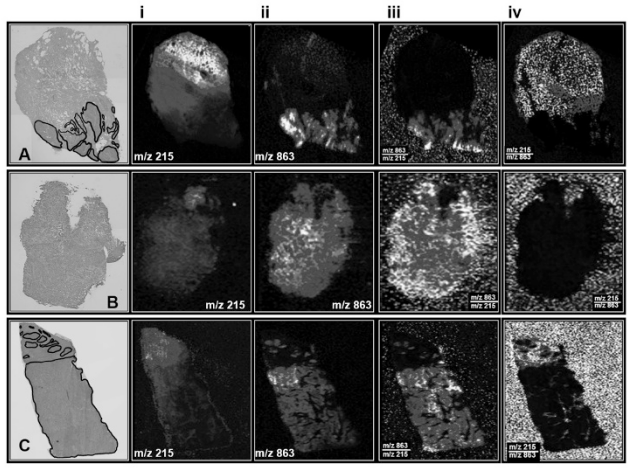

Fig 2. H&E histology and DESI MS images from invasive ductal carcinoma A-C showing the distribution of metabolites with *m/z 215* and *863* with increased relative abundance in non-neoplastic and tumor tissues respectively. DESI MS images of the ratio of *m/z 215 and 863 (iii)* showed better correlation with histology in its ability to detect tumor signals at tissue margins compared to single metabolite display. Tumor nests are contoured in black in A an C. Specimen B has diffuse infiltrative tumor not amenable to contouring.

Conclusions: Our findings demonstrate that DESI MSI based on the relative abundance ratio of selected metabolites in tumor versus non-neoplastic tissue has the potential to be an adjunct technique to histology for margin assessment. Additionally, it provides metabolic signatures to inform *in vivo* margin assessment techniques like 'iKnife' that our group is currently investigating.

257 Analysis of Ezrin and Phospho-Ezrin (Active) as Biomarkers of Invasion and Metastasis in Breast Cancer

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Background: As metastasis is the leading cause of mortality in breast cancer (BC), there is a need to identify novel biomarkers that predict the onset of metastatic disease. Ezrin, a member of the Ezrin-Radixin-Moesin (ERM) family of cytoskeletal cross-linker proteins, is frequently over-expressed in breast cancer and has been shown to promote cancer cell invasion and metastasis in preclinical models. However, there is limited understanding of ezrin expression pattern during BC progression.

Design: In this study we evaluated ezrin, active phospho-T567-ezrin (pTERM), and moesin expression by immunohistochemistry in a tissue microarray containing paired primary and metastatic invasive ductal carcinoma (IDC) cores (n=11). Furthermore, we examined ezrin expression in non-neoplastic, ductal carcinoma in situ (DCIS), and IDC regions of breast tumors (n=20) using a multiplexed quantitative immunofluorescence (mQIF)-based assay. Levels of membranous and cytoplasmic expression of biomarkers were scored by a pathologist using an automated image analysis algorithm (HALO™ software) to generate H-scores.

Results: mQIF analysis of primary tumor sections revealed a significant increase in ezrin expression in IDC and its leading-edge regions compared with DCIS and non-neoplastic tissue. (p=0.0003)

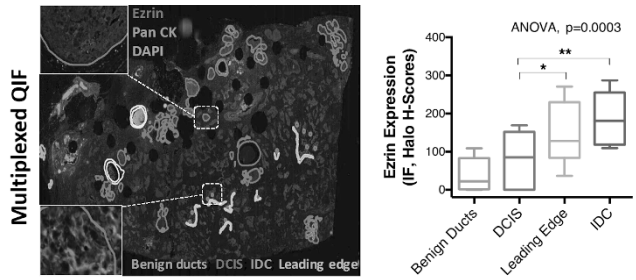

Fig 1. mQIF analysis of ezrin expression in primary breast tumor sections showing heterogeneity between benign ducts, DCIS, IDC and its leading edge.

In addition, we observed an increase in membranous expression of ezrin (p=0.05) and pTERM (p=0.02) in metastases compared with paired primary tumors from ER^+ patients (n=7).

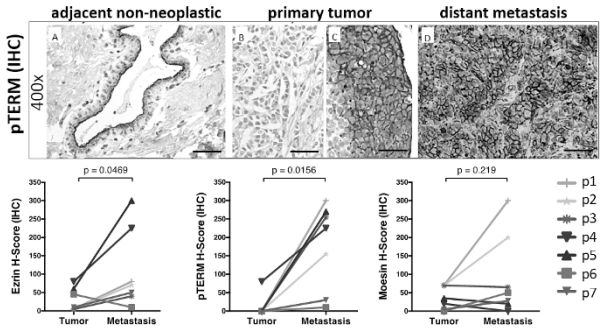

Fig 2. IHC analysis of ezrin, pTERM (phospho-ezrin/moesin), and moesin expression in non-neoplastic breast (A), paired primary (B, C) and metastatic breast tumors (D).

Moesin, also detected by pTERM antibody, did not show a significant increase from primary to metastatic tumors.

Conclusions: Findings presented in this study provide evidence that ezrin is the key ERM protein involved in BC invasion and metastasis in ER$^+$ patients and a potential biomarker and therapeutic target for metastatic disease.

258 Comparison of PD-L1, B7-H3, and PD-1 Expression in HIV Patients and Immunocompetent Patients with Breast Cancer

Stephanie Richards, Gary Rose, Paula Rosenblatt, David Riedel, Madhurima Koka, Kimberly Tuttle, Ashley Cellini, Olga Ioffe. University of Maryland School of Medicine, Baltimore, MD.

Background: Co-signaling molecules PD-1, PD-L1, and B7-H3 function in T-cell activation and self-tolerance. Research has shown that they may have an important function in tumor immunology, and may be targets for breast cancer therapy. Knowledge of the expression of these molecules in immunosuppressed patients with breast cancer is essentially absent, and thus the appropriateness of use of immune checkpoint inhibitors in these patients is uncertain.

Design: Our study analyzed 103 cases of breast cancer (16 with HIV, 87 immunocompetent). HIV patients were matched with immunocompetent patients by race (all African-American, AA) and tumor stage, which resulted in a subset of 64 immunocompetent AA patients. These 80 tumors stained for CD8, PD-1, PD-L1 and B7-H3. Tumor intrinsic subtypes were approximated by a combination of routine prognostic marker expression (ER, PR, HER2/neu and Ki-67, all by image analysis). The distribution of these subtypes was as follows in HIV patients: 2 tumors were triple negative (TNC), 3 luminal A (LumA), 8 luminal B (lumB) and 3 HER2-positive (HER2); the tumors in immunocompetent patients were 21 triple negative (TNC), 12 luminal A (LumA), 23 luminal B (lumB) and 8 HER2-positive (HER2). Stromal lymphocyte density (TILs), density of peri- and intratumoral CD8 T lymphocytes, and proportion of CD8-positive lymphocytes expressing PD-1 (PD-1+/CD8+) and PD-L1 (PDL-1+/CD8+). PD-L1 expression in tumors and lymphocytes was assessed by Aperio® image analysis.

Results: The proportion of TNC and high histologic grade was higher in the immunocompetent patients. B7-H3 expression was significantly higher (H score average 123 vs. 41, p=0.02) in immunocompetent vs. immunosuppressed patients, however there was no significant difference in the expression of other immune checkpoint molecules.

Conclusions: An interesting observation was the significantly smaller proportion of TNC in AA HIV patients, as compared to the group of age and stage-matched AA controls. This supports the notion that HIV infection does not portend a more aggressive tumor behavior. Although tumor B7-H3 expression was significantly higher in immunocompetent vs. immunosuppressed patients, the expression of other immune checkpoint molecules did not differ in the HIV patients. Our results provide reassurance for the use of checkpoint inhibitors against these co-signaling molecules in both immunocompetent as well as immunosuppressed patients although the significance of low B7-H3 expression needs to be further elucidated before such therapies are considered.

259 PD-1, PD-L1, and B7-H3 Expression in Intrinsic Molecular Subtypes of Breast Cancer

Gary Rose, Stephanie Richards, Paula Rosenblatt, Madhurima Koka, Kimberly Tuttle, Ashley Cellini, Olga Ioffe. University of Maryland School of Medicine, Baltimore, MD.

Background: Co-signaling molecules PD-1, PD-L1, and B7-H3 function in T-cell activation and self-tolerance; they have an important role in tumor immunology, and may be targets for breast cancer therapy.

Design: 103 cases of breast cancer were analyzed with immunohistochemistry for CD8, PD-1, PD-L1 and B7-H3. Tumor intrinsic subtypes were approximated by a combination of routine prognostic marker expression (ER, PR, HER2/neu and Ki-67, all by image analysis). 40 tumors were triple negative (TNC), 21 luminal A (LumA), 24 luminal B (lumB) and 16 HER2-positive (HER2). Stromal lymphocyte density (TILs), density of peri- and intratumoral CD8 T lymphocytes, and proportion of CD8-positive lymphocytes expressing PD-1 (PD-1+/CD8+) and PD-L1 (PDL-1+/CD8+). PD-L1 expression in tumors and lymphocytes was assessed by Aperio® image analysis. Statistical analysis was performed using linear regression, non-parametric Mann-Whitney test, chi-square test and Spearman's rank correlation.

Results: Histologic grade correlated with PD-L1 expression in tumors and TILs, with CD8 density and PD-1+/CD8+. Ki-67 index correlated with TILs, CD8 density, PD-1+/CD8+ and B7-H3 expression. PDL-1 was positive (defined as >5% staining and expressed as H score) in 26 tumors (25%); its expression was significantly higher in TNC and HER2 than luminal tumors. PD-1+/CD8 and +PDL-1+/CD8+ were higher in HER2 than other tumor subgroups. B7-H3 expression in tumor cells was often accentuated at the invasive edge; in addition a novel finding of positive staining of peritumoral angiogenic vessels was present in some tumors; both patterns significantly correlate with tumor grade and stage, Ki-67 index, TILs, CD8 density and PD-1+/CD8. B7-H3 expression was higher in TNC than other intrinsic subtypes.

Conclusions: Higher PD-L1 expression was observed in higher grade tumors and in aggressive breast cancer subtypes (TNC and HER2) as compared with subtypes with better prognosis (luminal A and B). Highest PD-1+/CD8 and +PDL-1+/CD8+ were seen in HER2 tumors. B7-H3 expression was highest in TNC. These results confirm the notion of higher immunogenicity of aggressive breast carcinomas, which lends further support for the use of immune checkpoints inhibitors in aggressive breast cancer. B7-H3 appears to be a molecule involved not only in immune signaling but its expression at the advancing tumor edge and in peritumoral angiogenic vessels in high-grade tumors is a novel finding and warrants further investigation of its clinical and therapeutic significance.

260 Digital Analysis of Tumour Microarchitecture as an Independent Prognostic Tool in Breast Cancer

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Background: Pathological assessment remains the gold standard for treatment decision making in breast cancer. As only a portion of the histological information is accessible by eye, recognition and quantification of complex patterns and relationships among constituents of the tumour microenvironment is feasible only with digital image analysis. This approach has the potential to exploit previously unquantifiable features as prognostic parameters. This paper builds on a previous small-scale study, and expands preliminary findings to a large cohort of patients with detailed histological and outcome data.

Design: Digitised keratin-stained tissue microarrays from 857 patients with invasive ductal carcinomas of no special type were analysed. Virtual slides were saved as ndpi files, captured as jpeg files and processed with a public domain image analysis software. Our aim was to assess the prognostic potential of tumour cell arrangement in variably sized groups (nests). The underlying working hypothesis was based on the the concept that tumour nest microarchitecture affects tumour-stroma interaction, and impacts tumour progression.

Results: We separately evaluated cases from the 4 major molecular subtypes. ER+ HER2- grade 2 tumours showed significant correlations between microarchitectural features (high nest number, low mean area and perimeter) and axillary lymph node (LN) involvement (p=0.004, 0.01, 0.001). These features were not correlated with tumour size. For other grades of this molecular subtype and all grades of other molecular subtypes, these features were not associated with LN status. Furthermore, from cases that presented with positive LNs, those with later distant metastasis (DM) had a lower mean nest area than those without subsequent distant metastasis. The difference was significant in grade 1 ER+ HER2- tumours and grade 3 ER- HER2+ tumours. Triple negative grade 3 tumours with initial LN involvement and subsequent DM showed a significantly higher number of nests than corresponding cases without DM.

Conclusions: Image analysis can tackle the difficulty of objective description and quantification of histological features, and promises to open up new horizons in histomorphometry. In this study, we showed that tumour microarchitectural features can be employed as prognostic markers for identifying breast cancer patients with increased metastatic potential. Our methodology has the potential to develop into a valuable clinical test.

261 Genomic Profiling of Pure Apocrine Carcinoma

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Background: Apocrine differentiation may be superimposed on a variety of breast cancer (BC) subtypes, and histologically defined apocrine
carcinomas demonstrate various biomarker profiles. Accordingly, "BC with apocrine differentiation" as defined by the WHO is a heterogeneous group. Pure apocrine carcinoma (PAC) has been applied to BC with apocrine cytology and a specific immunophenotype: ER/PR/HER2 negative (TN) and androgen receptor (AR) positive. It is unclear if PAC represents a more homogeneous entity with common molecular features. Furthermore, targeted therapy is not available for invasive PAC (iPAC) due to TN status. We profiled iPAC and associated apocrine ductal carcinoma in situ (ADCIS) using capture-based next generation sequencing (NGS) to gain insight into their pathogenesis and explore potential therapeutic targets.

Design: DNA was extracted from 11 PAC and 7 ADCIS with matched normal tissue from 12 patients. NGS was performed targeting coding regions of 510 cancer genes and 40 introns. Duplicate sequence reads were removed computationally for accurate allele frequency determination and copy number calling. Single nucleotide variants, insertions/deletions, and copy number alterations (CNA) were evaluated.

Results: Mean age was 67 (range 36-81). 11 patients had iPAC and ADCIS, and 1 had ADCIS and spindle cell carcinoma (SCC). Recurrently mutated genes included PIK3CA (6), PIK3R1 (6), PTEN (2), TP53 (4), HRAS (2), NF1 (2) and ERBB2 (2).

Pathogenic mutations in PIK3CA or PIK3R1 were present in 11/12(92%) and were mutually exclusive in 10; identical mutations were present in iPAC and ADCIS in cases with both components (n=6). 4 (33%) tumors had pathogenic RAS pathway mutations (2 HRAS, 2 NF1). In all cases with separately sequenced components, ADCIS and iPAC shared mutations, but divergent tumor evolution was also noted in 4; 3 of these had pathogenic mutations (PIK3CA, TP53, NF1) unique to iPAC and not in ADCIS. Activating ERBB2 hotspot mutations were present in 2 ADCIS, 1 of which had associated iPAC lacking the mutation. ADCIS and associated SCC shared hotspot TERT promoter and HRAS mutations, among others. Recurrent CNA included 6p gain (n=5) and loss of 6q, 9p and 17p (n=6 each).

Conclusions: Morphologically and immunophenotypically defined PAC represents a more homogenous subset of TNBC with near universal PI-3 kinase pathway activation. RAS pathway and ERBB2 mutations are also enriched in these tumors. ADCIS and iPAC show evidence of divergent tumor evolution, with NF1 and TP53 mutations associated with progression to invasion.

262 Correlation of Phosphohistone H3 Immunohistochemistry for Mitotic Count and Ki-67 Proliferation Index in 208 Cases of Breast Carcinoma

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Background: Mitotic index (MI) in the commonly used Nottingham Scoring System in breast cancer grading has been shown to be the most powerful prognostic factor in breast carcinomas; however, in daily practice measuring MI is highly subjective with only moderate interobserver reproducibility. Also, accurately observing mitotic figures can be obscured by technical artifacts, such as fixation times, overstraining with Hematoxylin, and distinguishing between mitoses and other changes, such as apoptosis and karyorrhexis. Accurate mitotic count requires a considerable amount of time and is highly influenced by the patience and diligence of the pathologist doing the counting. Ki-67 Proliferation Index (PI) has the potential to be used as a surrogate marker for mitotic activity; however, it will stain all the proliferating cells in G1, S, G2 and M phases of the cell cycle, thus overestimating the MI. Phosphorylation of histone H3 is a specific event in the mitotic phase and is negligible during interphase. Antibody against Ser10 of PHH-3 has been used to measure MI in many tumors so far.

Design: A Tissue Microarray (TMA) was built using 208 breast core biopsies of the patients with breast cancer diagnosis in the pathology archive of the authors' institution. Immunohistochemistry with MIB-1and PHH-3 antibodies using Ventana automated staining system was performed. TMA stained slides were scanned with Hamamatsu scanner at 40x magnification. Virtual slides were viewed by NDP viewer software to identify the hot spots for MI and PI. Two hundred cells were counted by the first author (a senior pathology resident) and percentages of positive nuclear staining for PHH-3 and Ki-67 were recorded. Statistical analysis was performed using SPSS software.

Results: PI ranged between 0 and 98 percent with the mean value of 32.3 and standard deviation of 24.28. While MI ranged between 0 and 23 percent with the mean value of 2.95 and standard deviation of 3.84. A significant correlation between Ki-67 proliferation index and PHH-3 mitotic activity was observed (Pearson correlation: 0.704, P<0.000).

Conclusions: Even though Ki-67 proliferation index is being used as a surrogate marker of proliferative activity in breast carcinomas, it has not been incorporated in any scoring system yet. The strong and statistically significant correlation between PHH-3 mitotic index and Ki-67 proliferation index proves the usefulness of PHH-3 immunohistochemistry in lieu of visual mitotic count on H&E stained slides.

263 Trop-2 Overexpression in the Axillary Lmph Nodes of Patients with Lobular Carcinoma Identifies Candidates for Adjuvant Treatment with the Antibody Drug Conjugate IMMU-132

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Background: Trop-2 is a cell-surface protein found to be overexpressed in several types of epithelial tumors. Recently, the antibody-drug conjugate sacituzumab goveitecan (IMMU-132) directed to Trop-2 was developed, tested and received "Breakthrough Therapy Designation" from the FDA after a successful phase II clinical trial for the treatment of triple negative breast cancer patients who had failed at least 2 prior therapies for metastatic disease. Using an immunoprofiling, discovery-based approach, we recently found metastatic, infiltrating lobular carcinoma (ILC) to exhibit strong membranous expression of Trop-2 in a singular case. In the current study, we have extended that query to a cohort of ILC with metastatic disease to determine if patients with this subtype of breast cancer are suitable candidates for treatment with IMMU-132.

Design: Ten cases of ILC with metastatic disease to axillary lymph nodes were identified from the archival files at the University at Buffalo's Department of Pathology. A block with lymph nodes possessing metastatic disease from each case was selected and stained with a polyclonal antibody to Trop-2 (Abcam) using conventional immunohistochemical techniques.

Results: The lymph nodes with metastatic ILC in all ten cases demonstrated circumferential membranous staining (CMS) for Trop-2 in the tumor cells. This finding was present in greater than 30% of the tumor cell population in 9 of the 10 cases. In the latter, >10% but <30% of the tumor cells exhibited CMS.

Conclusions: No definitive criteria currently exists for the selection of patients for treatment with IMMU-132. However, our findings of Trop-2 overexpression in a majority of tumor cells in the lymph nodes of patients with ILC suggest that patients that have this subtype of breast cancer with metastatic disease are excellent candidates for adjuvant treatment with IMMU-132.

264 Histological Findings in Benign Breast Biopsies After Tamoxifen Therapy for Breast Cancer

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Background: Women being followed up for breast cancer and treated with Tamoxifen (Tam) sometimes undergo breast biopsies in order to evaluate worrisome but ultimately benign radiographic or clinical abnormalities. The spectrum of histological findings in this subset of patients has not been well described.

Design: We reviewed archival formalin fixed paraffin sections in 52 women who underwent a benign breast biopsy during or after treatment with Tam for a contralateral breast cancer. Overall histological findings were classified as non proliferative (NP), proliferative without atypia (PDWA) or atypical hyperplasia (AH) based on standard criteria. Degree of lobular involution was also assessed and graded as none (<25% atrophic terminal duct lobular units {TDLU}), partial (26-75% atrophic TDLU) or complete (>75% atrophic TDLU).

Results: The mean age of the patients was 58 years (range: 38-77) at baseline and 64 years (range: 39-82) at follow-up benign biopsy. The median interval to benign biopsy was 4.5 years (range: 0.6-18 yrs). Fifty percent (26/52) patients were still taking Tamoxifen at the time of second biopsy, and another 12/52 (23%) underwent biopsies after completion of the recommended five years of therapy; thus, compliance was high at 73% overall. Biopsies most commonly showed proliferative histological features (44%, 23/52); PDWA lesions most commonly observed included columnar alteration/cell hyperplasia (65%), sclerosing adenosis (52%), and moderate to florid usual ductal hyperplasia (43%). However, a significant proportion was classified as atypical (21%, 11/52), with 9 showing ALH and 2 showing ADH. A very high proportion of samples were characterized by complete lobular involution (80%) at second biopsy as compared to 59% at baseline (p=0.02). Among 20 samples not showing complete involution at baseline, 11 (55%) showed progression of lobular involution by follow-up post-Tamoxifen biopsy, and importantly, women who were age 55+ at baseline showed progression by second post-Tam biopsy in 7/8 (88%).

Conclusions: The high frequency of proliferative changes and atypia suggest that Tam does not necessarily suppress emergence of, or eradicate, hyperplastic lesions. The degree of lobular involution in the subset of patients, however, suggests that Tam may induce atrophic histological changes in normal TDLU.

265 Prognostic Markers Are Relatively Stable Between Primary Breast Carcinoma and Metastatic Sites: A Retrospective Study

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Background: Studies on paired primary and metastatic sites report discordant rate of 15-40% for estrogen receptor (ER) and progesterone receptor (PR), and 7-26% for Her2. Switch in biomarker expression in breast metastases may affect the subsequent therapeutic approach. The goal of our study is to investigate any conversion of tumor markers between primary and metastatic sites, and evaluate the effect of treatment on these alterations.

Design: We retrieved 143 breast cancer patients with metastasis between 2011 and 2014, of which 119 patients had ER, PR and Her2 status available on both primary and metastatic sites. Primary tumors were categorized into 4 subtypes: Luminal [hormone receptor (HR) positive, Her2 negative], Her2+/HR+, Her2+/HR- and triple negative (TNBC). Treatments including chemotherapy (CT) and hormonal therapy (HT) were reviewed in all cases. Change of HR status was defined as complete loss of expression of both ER and PR, or gain of either ER or PR by IHC. Her2 status was based on either IHC and/or FISH.

Results: The discordancy for HR and Her2 between primary and metastases was 10% (12/119) and 1% (1/119), respectively (Table). Of the 12 discordant cases with change in HR, 10 were HR+ tumors with loss of expression in the metastases: 6 received both HT and CT, 4 received only CT. The other 2 cases were HR- tumors and both had weak ER expression in metastases. They both received CT after the primary diagnosis, but did not receive HT after metastasis. No HR expression loss in metastasis was observed in 9 patients who received only HT. Of the 23 Her2+ tumors, only 1 case with bone metastasis was negative for Her2 by IHC. No FISH analysis was performed. The specimen was decalcified. The patient had received CT including Herceptin after primary diagnosis, but did not receive Herceptin after the bone metastasis.

Type	No. of cases (%)	No. of discordant cases (%)	Type of discordancy	
			Change in HR (%)	Change in Her2 (%)
Luminal	68 (57)	7 (10)	7 (100)	0 (0)
Her2+/ HR-	12 (10)	2 (17)	1 (50)	1 (50)
Her2+/ HR+	11 (9)	3 (27)	3 (100)	0 (0)
TNBC	28 (24)	1 (4)	1 (100)	0 (0)
Total	119 (100)	13 (11)	12 (92)	1 (8)

Conclusions: The overall change in HR profile between the primary breast tumor and in the corresponding metastasis is low. Change in HR status is more likely related to chemotherapy than hormonal therapy. HER2 status remains relatively stable and any loss of HER2 requires confirmation by FISH. It is rare for triple negative breast cancer to change its profile.

266 Artefactual Displacement of Ductal Carcinoma In Situ (ADDCIS): A Mimicker of Invasive Carcinoma

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Background: Needle tract-associated epithelial displacement is a recognized mimicker of invasive mammary carcinoma (IMC). Artefactual displacement of ductal carcinoma in situ (ADDCIS) unassociated with prior needle biopsy may occur secondary to mechanical compression of breast specimens; however, this phenomenon has not been formally studied.

Design: We identified 14 cases of ADDCIS from our files that were not associated with macroinvasive mammary carcinoma elsewhere. Clinical and histologic features were reviewed.

Results: Of 14 cases of ADDCIS, 1 (7%) had been diagnosed as IMC at an outside hospital (OH), 3 (21%) were true consultations from OHs to rule out IMC, and 10 (71%) were referred by general pathologists at our hospital to the breast pathology service to rule out IMC. The median patient age was 57 years (range 44-74). Eight (57%) ADDCIS occurred in lumpectomy specimens, 4 (28%) in mastectomy specimens, and 2 (14%) in core biopsy specimens. The average DCIS size was 2.2 cm (range 0.4-6.5 cm); 1 DCIS case had a separate focus of microinvasion. DCIS nuclear grade was 1 in 3 cases (21%), 2 in 5 cases (36%), and 3 in 6 cases (43%). ADDCIS size ranged from <1mm to 5mm; all 4 cases of ADDCIS ≥4mm had a linear pattern of displacement. In all cases the ADDCIS cells were unassociated with stromal reaction and showed degenerative, smudged chromatin compared to adjacent nondisplaced DCIS. Immunohistochemistry revealed no myoepithelial cells around ADDCIS cells in all 6 cases in which it was performed. Four (29%) ADDCIS cases were associated with a papillary neoplasm. None of the 9 patients with follow-up developed metastasis (mean follow-up, 7 years; range 4-11 years). All received further local therapy for DCIS (radiation in 5 cases, completion mastectomy in 4) but only one received adjuvant systemic therapy (hormone therapy following a subsequent contralateral IMC).

Conclusions: ADDCIS is an underappreciated mimicker of IMC, particularly given the absence of myoepithelial cells around the displaced DCIS cells. Unlike needle tract-associated displacement, ADDCIS is not strongly associated with papillary neoplasms. While most commonly found in lumpectomies with DCIS, ADDCIS can be seen in mastectomies or core biopsies. Diagnostic clues include the smudged nuclear chromatin of the displaced DCIS cells compared to adjacent DCIS, the lack of stromal response, as well as the linear pattern of displacement. The benign follow-up in the absence of systemic therapy supports our view that ADDCIS does not represent true IMC.

267 Clinicopathologic Review of Non-Classic Lobular Carcinoma In Situ Variants at a Single Academic Center

Eliah Shamir, Tianming Chu, Joseph T Rabban, Yunn-Yi Chen, Gregor Krings. UCSF, San Francisco, CA.

Background: Pleomorphic (PLCIS) and florid lobular carcinoma in situ (FLCIS) are rare variants (LCISv), considered to be more aggressive than classic LCIS (cLCIS) and likely non-obligate precursors to invasive carcinoma. However, natural history, outcomes and treatment data of these lesions remains very sparse, and optimal treatment consensus is lacking. We analyzed clinicopathologic features, outcome and treatment of LCISv present in core biopsy (CB) and/or surgical excision (EXC) at a large academic center in order to help guide management.

Design: Clinical, radiologic and pathologic findings from all LCISv cases diagnosed on CB and/or EXC over 23 years were reviewed with focus on demographics, presentation, associated lesions (cLCIS, ductal carcinoma in situ [DCIS], invasive carcinoma [IC]), margins and treatment.

Results: Mean age was 60 (range 35-86). Of 86 LCISv, 58 (67%) were PLCIS and 28 (33%) were FLCIS. Ten (12%) were associated with DCIS and 61 (71%) with IC (29 ILC, 23 pleomorphic ILC [PILC], 3 IDC and 6 IC with ductal and lobular features [IDLC]); 15 (17%) were pure LCISv without IC. Of all patients with pure LCISv and follow-up (FU; n=11), none received radiation and 50% endocrine therapy. Final pLCISv margins were positive in 1 case. No pure LCISv recurred (mean FU 52 months). All pure LCISv on CB represented the targeted lesion (n=19). Ten (50%) presented as calcifications (calcs); only 1 presented as palpable mass and was PLCIS in a sclerosing lesion. In 84%, cLCIS was also present. 91% pure PLCIS and 50% pure FLCIS on CB underwent surgery; the upgrade rate was 26% (1 DCIS, 1 PILC, 3 ILC).

				LCISv+IC			
	Patients	Pure LCISv	LCISv +DCIS	microinvasive ILC	ILC, PILC	IDC	IDLC
PLCIS+/- necrosis (nec) (n)	52	5	6	1	13,23	2	2
Apocrine PLCIS+/- nec (n)	6	6	0	0	0,0	0	0
FLCIS-nec (n)	12	3	1	1	5,0	0	2
FLCIS+nec (n)	16	1	3	4	5,0	1	2
Total (n)	86	15	10	6	23,23	3	6

CB Diagnosis(Dx), Pure LCISv	Patients	Excision (Ex)	Mastectomy (M)	M after Ex for close/+ margins	# upgraded	Upgrade Dx
PLCIS+/-nec (n)	6	1	2	2	3	PILC(1),ILC(2)
Apocrine PLCIS+/-nec (n)	5	3	1	1	0	-
FLCIS-nec (n)	3	0	0	0	0	-
FLCIS+nec (n)	5	1	1	2	2	ILC(1), DCIS(1)
Total (n)	19	5	4	5	5	

Conclusions: Most LCISv are associated with IC, most often ILC or PILC. The upgrade rate of pure LCISv on CB to IC on EXC is 26%. In contrast to pure cLCIS, pure LCISv on CB is typically the targeted lesion and not incidental. No women with pure LCISv at our center received radiation, but endocrine therapy was common. No pure LCISv recurred.

268 Prognostic Outcomes in Advanced Breast Cancer (BC): The Metastasis-Free Interval (MFI) Is Important

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Background: Metastatic BC is a heterogeneous disease with a diverse clinical course. Despite the recent improvement in early detection and aggressive treatment, 5 to 10% of patients are diagnosed with Stage IV BC at presentation (de novo metastatic BC) and approximately 20 to 30% will develop metastatic recurrence during follow-up. There have been controversial findings reported regarding prognostic outcomes in regard to this group of patients with advanced BC. This study was thus aimed in determining the potential prognostic impact of the time of metastatic presentation.

Design: Advanced BC cases seen at the authors' institution were identified from 1998 to 2013. The clinicopathologic parameters of the primary BC along with therapeutic modalities applied and outcomes achieved were recorded. Analysis of overall survival (OS) after the diagnosis of first distant metastasis was performed using both the Kaplan-Meier method and the log-rank test.

Results: Of the 572 patients with advanced BC in the study period, 492 (86%) received systemic therapy. Factors significantly associated with OS after distant metastasis in this group included race, histologic grade, subtype, the number of organs involved, and the time of metastasis. Being Caucasians and harboring a Grade I/II BC were associated with a prolonged OS. Triple-negative BC was associated with a worse outcome compared to the luminal and HER2 subtypes, whereas no significant difference was found between the latter two. Isolated metastasis had a significant survival benefit over multi-organ involvement, while no preferable metastatic site was identified among the common organs of relapse (bone, liver, lung and brain). Interestingly, de novo metastatic BC was associated with a significantly prolong OS compared to metastatic recurrence with a MFI <2 years, but did not significantly differ from that with a MFI >2 years. Importantly, de novo metastatic BC was associated with a worse prognosis compared to recurrent metastatic BC in the 80 patients who did not receive systemic treatment, regardless of MFI. This is in contrast to the findings in a recent study in which the association between MFI and survival was seen irrespective of the use of systemic therapy [Br J Cancer. 2015;112(9):1445-51].

Conclusions: The findings provide further insight into the natural history of advanced BC. De novo metastatic BC demonstrated a better response to systemic therapy compared to the subset of recurrent metastatic BC with a shorter MFI and thus might be regarded as a separate entity. MFI is a strong prognosticator in the progression of relapsed BC.

269 Low Frequency of HER2-Positive Male Breast Cancers

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Background: Mammary carcinoma in men is rare, accounting for less than 1% of all breast cancers. Given the relatively limited data available on male breast cancers, the clinicopathological features of 40 consecutive primary invasive mammary carcinomas (n=40) and 5 ductal intraepithelial neoplasias (ductal carcinoma in situ) were reviewed.

Design: Clinicopathological features of 45 consecutive invasive and/or intraepithelial mammary neoplasias (carcinomas) diagnosed over the past 10 years in our institution were reviewed. All 40 invasive carcinomas had ER, PR, HER2 (IHC) and HER2 (FISH) assessment. All 5 ductal intraepithelial neoplasia (DIN) cases underwent ER, and PR testing. Additionally, 14 cases (12 invasive carcinomas and 2 DIN cases) underwent AR testing.

Results: The average age of patients with invasive carcinoma was 67 years (range 42-89, median 69). The average age for DIN cases was 60 (range 46-72, median 63). All invasive and intraepithelial lesions were of ductal type. No lobular neoplasias were identified. Most of the invasive carcinomas were well to moderately differentiated (70%), while 30% were poorly differentiated. Focal mucinous features were present in one case, and micropapillary features were noted in another case. DIN (DCIS) was seen in 45% of cases with a papillary DIN noted in 5% of the cases adjacent to the invasive carcinoma. All 40 invasive carcinomas were ER-positive (100%), while 36 of them (90%) were PR-positive. Mean H-score for ER was 260 (range 60-300). Mean H-score for PR was 180 (range 0-300). Out of three HER2-FISH amplified cases, one was 3+, the remaining two cases were 2+ equivocal on immunohistochemistry. All of the 12 cases tested for AR were positive. Only 3 of 40 cases (7.5%) were amplified for HER2-FISH. Sixty percent of DIN (3 of 5 cases) cases displayed papillary architecture

(papillary DIN) and all 5 cases were ER and PR positive. AR performed in two DIN cases was also positive. There was no triple negative case including metaplastic carcinoma or adenoid cystic carcinoma.

Conclusions: The frequency of HER2-amplification in male breast cancers is lower than (about half) that of female breast carcinomas, while all those tested for ER and AR were positive. We had no cases of lobular neoplasia, either invasive or intraepithelial. As noted previously, a high proportion of pure intraepithelial (in situ) lesions had a papillary architecture.

270 Cost Effectiveness of Intraoperative Examination of Sentinel Lymph Nodes in Patients with DCIS Compared to Invasive Breast Carcinoma

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Background: Preliminary results for a sentinel lymph node biopsy (SLNB) can be obtained intraoperatively via frozen section to guide whether an axillary lymph node dissection (ALND) is performed concurrently. There is no standard protocol for the use of intraoperative frozen section analysis of SLNs in patients with ductal carcinoma in situ (DCIS). Variability in the use of frozen sections has not been investigated and there is minimal information regarding the cost effectiveness of this technique in DCIS. We sought to investigate the usage, expenditures, and clinical impact of SLNB frozen section analysis in DCIS and compared these findings to invasive mammary carcinoma in a single large academic tertiary center.

Design: Pathology reports were retrospectively searched for breast resection specimens with SLNs submitted from 2012-2013. Medicare allowable billing rates for the relevant Current Procedural Terminology codes were used to estimate the pathology healthcare expenditures of all intraoperative frozen sections that were performed.

Results: 897 specimens from 885 patients were performed by 6 surgical oncologists (prior biopsy of DCIS only, n=151; prior biopsy of invasive mammary carcinoma, n=746). Rates of SLN frozen section requests per surgeon ranged from 52.8-100.0% but there was uniform use of frozen sections in mastectomy procedures for both DCIS (99%) and invasive tumor (99%). For the cohort, an estimated cost of $188,875.59 was calculated for 2252 SLN blocks frozen (DCIS, $39,464.24; invasive carcinoma, $149,411.35). Based on the number of positive frozen SLN diagnoses that resulted in synchronous ALNDs, $19,732.12 was spent to impact the surgical management of a single patient with DCIS (n=2) while only $1,624.04 was spent to impact the surgical management of a single patient with invasive carcinoma (n=92).

Conclusions: There was a substantially higher additional SLN frozen section cost to impact the surgical management of DCIS patients compared to patients with invasive carcinoma. Decreasing the use of routine SLN frozen sections in this setting represents an opportunity for reducing pathology expenditures.

271 Fibroepithelial Lesions of the Breast Involved by Atypical Epithelial Proliferations: A 12-Year Single Institution Study

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Background: Ductal carcinoma in situ (DCIS), atypical ductal hyperplasia (ADH), lobular carcinoma in situ (LCIS) and atypical lobular hyperplasia (ALH) rarely involve fibroadenoma (FA) or phyllodes tumor (PT). Case reports of this unusual combined lesion have been published, but to our knowledge there are no retrospective, longitudinal studies that have characterized their biology or clinical outcomes. We report a series of 30 cases from a single institution over a 12-year period.

Design: With IRB approval, we performed a search from 2004 – 2016 for all breast biopsies and excisions that contained a fibroepithelial lesion involved by DCIS, ADH, LCIS, or ALH. Clinical data were collected, including patient age, prior histories, type of surgical procedures, and follow-ups. Pathologic data collected for each case included tumor size, type of fibroepithelial lesion (FA or PT), type of atypical epithelial proliferation involving the lesion, extent of the atypical epithelial proliferation, and other histologic findings outside of the index lesion.

Results: Three cases of PT and 27 cases of FA were identified. The average patient age was 50 years (ranging 21-76 years). Of the 27 FA, 17 (63%) were involved by LCIS or ALH, and 10 (37%) were involved by DCIS or ADH. Of the 3 PT, 1 was involved by LCIS and 2 were involved DCIS or ADH. In 16 cases, excision was performed after the combined lesion was initially diagnosed on core biopsy. None of those 16 cases showed upgrades to invasive carcinoma on excision. In 2 cases, the combined lesion was seen only in the excision specimen, after a core biopsy demonstrated FA in one case and LCIS in another case. In 6 cases, excision was performed upfront after the clinical presentation of a breast mass with no prior core biopsy. Finally, in 6 cases, the combined lesion was seen on core biopsy but follow up excision data was not available.

Conclusions: The epithelial component of fibroepithelial lesions is rarely involved by atypical epithelial proliferations. There were no upgrades on excision when such combined lesions were first diagnosed on core biopsy. This finding implies that these atypical epithelial proliferations might behave differently within fibroepithelial lesions. Our series also suggests that lobular neoplasia occurs within FAs at a higher rate compared to ductal neoplasia. Because lobular neoplasia is in general less common than ductal neoplasia, this observation raises the possibility of a biological link that merits further investigation.

272 Inter-Observer Variability in Diagnosing Lobular Carcinoma-In-Situ Subtypes; Evaluating Reproducibility of Contemporary Criteria

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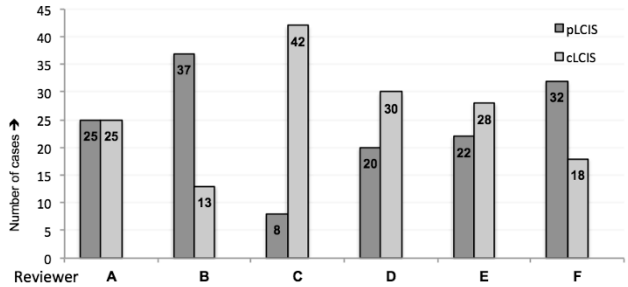
Background: Lobular carcinoma in situ(LCIS) subtypes include classical- (cLCIS), pleomorphic- (pLCIS) and florid-type (fLCIS). Current WHO classification provides morphological criteria for these diagnoses. Accurate diagnosis of LCIS depends upon recognition of subtypes: NCCN guidelines suggest that pLCIS management should be more aggressive and treated as ductal carcinoma in situ, while other LCIS subtypes are managed more conservatively. Inter-observer variability in diagnosing LCIS subtypes, using contemporary criteria, is not known. We aimed to evaluate inter-observer variability amongst breast pathologists in diagnosing LCIS subtypes.

Design: Six breast pathologists with variable 1 to > 25 years of experience independently reviewed 50 H&E stained slides comprised of a mix of LCIS subtypes. Provided to all was a copy of the WHO 2012 of LCIS subtypes criteria. Participants diagnosed pLCIS, cLCIS ± apocrine change in a 5 mm diameter marked region of interest and fLCIS based on entire section. **κ** statistic(Cohen & Fleiss) was applied as appropriate.

Results: Reviewers A-F diagnosed pLCIS in 25(50%), 37(74%), 8(16%), 20(40%), 22(44%) & 32(64%) slides respectively(Fleiss κ =0.558; moderate agreement), fLCIS in 22(44%), 30(60%), 18(36%), 22(44%), 26(52%) & 16(32%) slides respectively(Fleiss κ =0.687; substantial agreement) and apocrine cLCIS in 20(40%), 13(26%), 11(22%), 15(30%), 37(74%) & 23(46%) slides respectively(Fleiss κ =0.577; moderate agreement). Complete agreement for pLCIS, fLCIS and apocrine cLCIS was present in 4, 11 and 3 slides. Statistically significant agreement(Cohen κ; p<.05) was present in 4/15, 4/15 and 9/15 reviewer pairs for pLCIS, apocrine cLCIS and fLCIS respectively.

Figure 1. pLCIS and cLCIS diagnoses by all reviewers (top). Pairwise κ statistic for pLCIS (bottom)



Reviewer	A	B	C	D	E	F
B	.20					
C	.00	.125				
D	.24	.161	.259*			
E	.16	.054	.303*	.18		
F	.28*	.458*	-.066	.046	.028	

*Significant κ (p<0.05).

Conclusions: Our study shows moderate agreement in diagnosing pLCIS, which may be suboptimal for patient management. Standardization of diagnostic cytologic features of pLCIS and apocrine cLCIS may improve reproducibility. fLCIS as an architectural pattern appears to be distinct and shows substantial interobserver agreement.

273 Does Pseudoangiomatous Stromal Hyperplasia (PASH) Exhibit Loss of 13q14?

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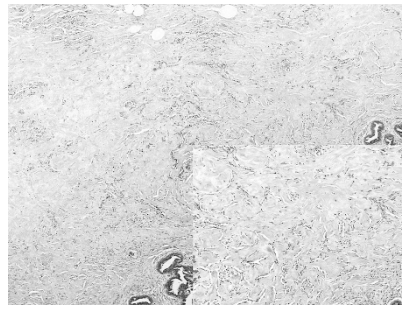
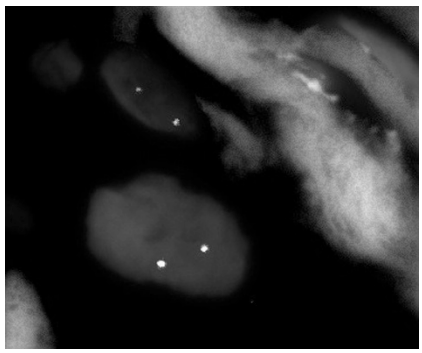
Background: Pseudoangiomatous stromal hyperplasia (PASH) is a benign myofibroblastic proliferation of the breast that can present clinically as a palpable mass or as an incidental finding. Microscopically, PASH is comprised of slit-like, often anastomosing, spaces lined by myofibroblasts in a dense collagenous stroma.



Historically, PASH and myofibroblastoma are thought to represent a spectrum of related myofibroblastic lesions, often with considerable overlapping morphologies. As monoallelic loss of 13q14 has been described in myofibroblastoma, and the related entities of cellular angiofibroma and spindle cell/pleomorphic lipoma, it seems plausible this molecular alteration may be present in PASH; however, to our knowledge this has not been previously studied. Herein, we describe a small study to determine if PASH shares monoallelic loss of 13q14 with the morphologically related entity of myofibroblastoma.

Design: A retrospective search for cases of PASH at a tertiary care center was performed. Seventeen cases, collected between 2004 and 2013, were identified, including both core biopsy and resection specimens. A single, most representative, block was selected after review of each case, and unstained, de-identified paraffin sections were obtained. Fluorescence in situ hybridization (FISH) using the Vysis FOXO1 Dual Color Break Apart Probe (13q14) was performed.
Results: In all 17 cases, FISH revealed normal signal patterns and failed to demonstrate monoallelic loss of 13q14.



Conclusions: Although PASH can show overlapping histologic features with myofibroblastoma, it does not appear that these entities share common molecular alterations. These findings could prove useful clinically when confronted with an indeterminate myofibroblastic lesion, as the results of FISH using the Vysis FOXO1 Dual Color Break Apart Probe (13q14), used in the diagnosis of alveolar rhabdomyosarcoma, could help differentiate between PASH and myofibroblastoma.

274 High Tumor Budding Count Is Associated with Poor Clinicopathological Features and Prognosis in Breast Carcinoma

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Background: Tumor budding (TB) is associated with adverse clinicopathological features and outcome in colorectal carcinoma. Its significance in breast carcinoma has not been well studied.
Design: A total of 244 cases of estrogen receptor-positive (ER+)/HER2-negative (HER2-) and 131 cases of triple-negative breast carcinoma (TNBC) diagnosed from 2004 to 2014 were included in this study. TB was defined as a cluster of < 5 tumor cells at the invasive front and evaluated at 200x high-power field (HPF). TBs were counted by 5 HPF at the hotspot identified on a low-power field (40x). The highest single TB count (H-TB) and average TB count (A-TB) in 5 HPFs were evaluated and correlated with lymph node metastasis (LNM), lymphovascular invasion (LVI), local recurrence, distant metastasis (other than axillary lymph node metastasis), overall survival (OS), and disease-free survival (DFS).
Results: In ER+/HER2- breast cancer, both H-TB and A-TB were significantly associated with distant metastasis but not other parameters in both univariate and multivariate analyses (all $P<0.05$). In TNBC, H-TB (but not A-TB) was associated with distant metastasis by univariate analysis but not multivariate analysis; and both H-TB and A-TB were associated with LVI and worse OS time by univariate and multivariate analyses (all $P<0.05$).
Conclusions: TB is associated with adverse clinicopathological features in ER+/HER2- and TNBC breast cancers. Evaluation of H-TB only may be sufficient in breast carcinoma.

275 Leptin-Receptor Expression in Luminal-Type Breast Carcinoma Is Associated with Pathologic Features of Diffuse Risk but Not with Tumor Proliferation

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Background: Obesity is associated with increased breast-cancer specific mortality in estrogen receptor (ER) positive disease, through unclear mechanisms. Leptin is a multi-functional protein with a key role in maintaining body weight through action on adipose tissue. Pre-clinical studies show that leptin stimulates growth, survival, and progression of breast tumor cells through both estrogen dependent and estrogen independent (e.g. JAK/STAT, PI3K/Akt/MAPK) pathways. Our aim was to correlate tumor leptin-receptor (OB-R) expression with histopathologic features of disease.
Design: We identified ER+/HER2- breast cancers (N = 122) with slides/reports available for review. Clinicopathologic data available included BMI, menopausal status, family/cancer/medical history, lymphovascular invasion, presence of DCIS/LCIS, and tumour stage. Immunohistochemistry for OB-R (polyclonal, 1:50) and Ki-67 were performed. OB-R was scored using the semi-quantitative Allred scale (0 to 8, % and intensity recorded). Ki-67 was scored as % by manual count. We reviewed H&E slides for patterns of tumor cell morphology, tumour growth, and stromal features. OB-R and Ki-67 expression were correlated with histopathologic features using descriptive statistics.
Results: All 122 tumors expressed OB-R (range: Allred 3 to 8; median 7, mean 6.61), with 109/122 (89%) having high expression (Allred ≥6). OB-R scores correlated with patient BMI and age (Pearson $r=0.78$ and 0.21, $p<0.00001$ and 0.023 respectively). Only OB-R-high cases were associated with the presence of LVI (22/109, 20%) or extensive DCIS (12/109, 11%). There was no significant difference between OB-R-high and OB-R low tumors with respect to tumor borders, tumour grade, stromal response, presence of LCIS, cytoplasmic or nuclear morphology, mitotic score, or Ki-67 index.
Conclusions: OB-R is ubiquitously expressed in ER+/HER2- breast tumor tissue but does not parallel tumor-cell proliferation. OB-R is associated with pathologic features of local recurrence (extensive DCIS, LVI). Together with preclinical data, these findings suggest that obesity leads to tissue-specific metabolic signaling, conferring risk independent of intrinsic tumor growth. Further work is needed to elucidate the network of interactions between hormone- and metabolism-dependent signaling in tumor progression.
(Funding: Hold'Em for Life)

276 Application of the 2013 HER2 FISH ASCO/CAP Guidelines: A New Hope for Breast Cancer Patients in the Philippines

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Background: HER2 (aka C-erb-B-2) is an oncogene & a type of human epidermal growth factor essential in the growth & development of certain types of breast carcinomas. This protein has been used effectively as a prognostic indicator and target of therapy in the management of breast cancers. With the application of the 2013 ASCO/CAP guidelines, women in the Philippines previously unqualified for treatment may now have another chance in their fight against the said disease.
Design: In this retrospective study, we reviewed previous HER2 FISH test results in our institution from Jun 2010-Nov 2013. During this period, the 2007 ASCO/CAP guidelines were utilized. We then compared it to the 2013 guidelines & tabulated into 3 categories which were negative, equivocal, & positive. Anonymity of the subjects was strictly observed.
Results: 350 HER2 FISH test results reviewed from Jun 2010-Nov 2013 revealed 41 (11.71%) cases changing to a different category when the 2013 guidelines were applied. From the 41 cases, 28 of which shifted from negative to equivocal, 12 from negative to positive, and 1 from equivocal to positive. 242 remained negative, 1 remained equivocal, & all 2007 HER2 FISH positive cases also remained unchanged.

FISH Results	Change in CategoryRe-evaluation using 2013 HER2 Guidelines	
Negative	40(11.43%)	From Negative to Equivocal 28(8.0%) From Negative to Positive 12(3.43%)
Equivocal	1(0.28%)	From Equivocal to Positive 1(0.28%)
Positive	0(0)	All 2007 HER2 FISH positive remained positive
Total	41(11.71%)	

There is a significant effect on the shift of the guidelines in changing HER2 status from negative to equivocal & negative to positive. It also affects those who will benefit from the therapy.

FISH Results	2007 HER2 FISH ASCO/CAP Guidelines	2013 HER2 FISH ASCO/CAP Guidelines	Percent Change
Negative	282	242	-14.18%
Equivocal	2	29	1,350%
Positive	66	79	19.70%
Total	350	350	

Conclusions: Cases of HER2 equivocal and HER2 positive results which were then either negative or equivocal increased significantly with the application of the 2013 HER2 FISH ASCO/CAP guidelines. With this outcome, 41 more previously diagnosed breast cancer patients in the Philippines may now take advantage of this therapy. Thus, more applicants, as well as previously denied patients, can qualify as candidates and benefit from this targeted therapy.

277 Morphological and Genomic Heterogeneity in a Malignant Phyllodes Tumor

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Background: Malignant phyllodes tumors (PTs) are rare biphasic fibroepithelial neoplasms of the breast with potential for clinically aggressive behavior, including recurrence and distant metastases. Treatment options are currently limited. Recent work has uncovered recurrent genetic aberrations in fibroepithelial lesions (FELs), which may prove to be actionable therapeutic targets. We performed exome sequencing on an unusual case of malignant PT with intratumoral heterogeneity and stromal keratin expression, which suggested divergent differentiation into metaplastic carcinoma.
Design: Tumor regions exhibiting morphological heterogeneity, i.e. uniform spindled cells in chondromyxoid stroma, storiform spindled cells with occasional rhabdoid cells, and pleomorphic cells with intermixed large rhabdoid cells, were selected for analysis. Formalin-fixed paraffin-embedded tissue sections were subjected to macro- and laser micro-dissection, the latter in order to separate epithelial and stromal tumor components. Whole-exome sequencing was performed on purified genomic DNA samples from each region. Variants were filtered to retain only those covered by at least 15 reads and having at least three variant reads. Variant allele frequencies lower than 5% and insertion/deletion mutations (indels) overlapping simple-repeat regions were excluded. All candidate variants were visually inspected in the Integrative Genomics Viewer genome browser to exclude likely germline mutations and sequencing artefacts.
Results: Sequencing revealed mutations in all regions of tumor stroma, including DNA repair, cell signalling, cell cycle regulating, and tumor suppressor pathway-related genes such as MED12, TP53, TRIP12, CDK4 and RBM10. Intratumoral genomic

heterogeneity was observed, with increasing numbers of mutations appearing to parallel morphological attributes of increasing cellularity and pleomorphism. A low frequency of mutations was found in tumor epithelium, which may be due to inadvertent contamination by tumor stromal cells.
Conclusions: Genomic aberrations correlate with stromal morphological heterogeneity in malignant PT, with implications for novel therapeutic modalities, including genomic targeting of metastatic foci. Further work on epithelial-stromal interactions in FELs will contribute to understanding of the pathogenesis of these unusual tumors.

278 Genomic Alterations in Breast Fibroadenomas and Phyllodes Tumors – Preliminary Findings from the International Fibroepithelial Consortium

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Background: Fibroepithelial tumors (fibroadenomas, phyllodes tumors) harbor recurrent genomic aberrations that are potentially important in pathogenesis and biological behavior. Mutations in the MED12 gene are reported in about 60% of fibroadenomas and phyllodes tumors (PTs), with alterations in TERT, RARA observed in the latter. Abnormalities in cancer driver genes appear in borderline and malignant PTs. While similar data has been described by various groups, no large scale study validating the role of these genes in tumors from diverse populations has been conducted.
Design: Participating institutions from the International Fibroepithelial Consortium contributed 1237 fibroepithelial tumors. Formalin fixed paraffin embedded samples were used for genomic DNA extraction. A custom panel of 16 genes was synthesized covering all exons except *TERT* with amplicons spanning promoter positions chr5:1295228 and chr5:1295250. Each nucleotide position was covered by a minimum of 2 overlapping amplicons. Sequencing was performed with the illumina Hiseq platform. Point mutations & indel detection were performed with Freebayes v0.9.20-16-g3e35e7210 and restricted to targeted amplicon regions. All samples were sequenced to a depth of greater than 200x of the target regions.
Results: Of 336 samples evaluated, *MED12* and *TERT* mutations were discovered in 54% and 37% of all fibroepithelial tumors. *TERT*, *RARA*, *SETD2* aberrations were observed more frequently in PTs than fibroadenomas. Malignant PTs disclosed *NF1*, *Rb1*, *TP53*, *PTEN*, *EGFR* mutations. Frameshift, nonsense, inframe indel, promoter, missense, splice site mutations were seen.
Conclusions: Preliminary findings from an international cohort of fibroepithelial tumors confirm recurrent mutations in *MED12*. *TERT*, *RARA* and *SETD2* aberrations are discovered in PTs. Malignant PTs demonstrate cancer driver mutations, lending support to the proposed progression model of fibroepithelial tumorigenesis.

279 Characterization of GATA3 Expression in Invasive Breast Cancer-Differences in Histological Subtypes and Immunohistochemically Defined Molecular Subtypes

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Background: GATA-binding protein 3 (GATA3) is a sensitive and relatively specific biomarker in breast and urothelial carcinomas. Its diagnostic utility in primary and metastatic breast cancers has been explored and confirmed. However, the relationship between GATA3 expression and different breast carcinoma intrinsic subtypes has not been specifically defined in the literature despite sparse reports with small case size. The aim of the current investigation is to clarify the GATA3 distribution among different histological subtypes and surrogate molecular breast carcinoma subtypes with a large series with comparison with the traditionally used breast cancer markers GCDFP15 and mammaglobin.
Design: Immunohistochemical staining of GATA3, GCDFP15 and mammaglobin was performed in a cohort of 1642 cases of primary invasive breast carcinoma. The association of GATA3 expression with different histological and surrogate intrinsic subtypes was assessed, also with a comparison with GCDFP15 and mammaglobin.
Results: The overall positivity of GATA3 across the various immunohistochemistry-based surrogate intrinsic subtypes is 99.51% for luminal A-like, 97.71% for luminal B-like, 68.50% for HER2 overexpression and 20.16% for triple negative breast cancers, respectively. GATA3 expression is positively correlated with ER positive (luminal subtypes) breast carcinomas. For luminal-like and HER2 overexpression subtypes, GATA3 is much more sensitive than GCDFP15 and mammaglobin. For triple negative tumors, GATA3 is less sensitive than GCDFP15.
Conclusions: GATA3 exhibits a pretty high sensitivity for breast carcinomas. It is more sensitive than GCDFP15 and mammaglobin in luminal-like and HER2 overexpression subtypes. GATA3 expression was associated with breast carcinomas of luminal subtype and low histological grade.

280 BRD4 Expression in Lymph Node Negative Breast Cancer: Correlation with T-bet+ Tumour Infiltrating Lymphocytes and Disease Free Survival

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Background: BRD4 (Bromodomain-Containing Protein 4) regulates gene transcription and is critical for cell cycle progression. Inhibitors of BRD4 have proven to be efficacious in many preclinical cancer models. We have shown that T-bet+ tumor infiltrating lymphocytes (TILs) are associated with poor prognosis features including the basal phenotype in breast cancer (BC) and yet, are associated with a favorable outcome. On examination of mRNA expression differences in T-bet+ versus T-bet- tumors, we identified BRD4 to be expressed at ~44 fold higher levels in T-bet+ tumors. We therefore examined the immunohistochemical (IHC) expression of BRD4 and T-bet in a large cohort of women with lymph node negative (LNN) BC and correlated it with clinicopathologic parameters and outcome.
Design: Using the Allred score, the IHC expression of tumoral BRD4 was assessed on tissue microarrays constructed from tumors from a prospectively accrued series of women with LNN BC. BRD4 data were available for 612 women. Hormone receptors (HR), 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. A cut-point of 6 was used to determine BRD4 positivity. Chi-square or Fisher exact test were used to analyze BRD4 associations with clinicopathologic variables, IHC markers, and molecular subtype. Survival analyses were by the log rank test and KM plots. All tests were two sided. A test with a P value <0.05 was considered statistically significant.
Results: 76.6% of tumors were BRD4 positive. BRD4 positivity was significantly associated with T-bet+ tumors (P=0.036) , pre-menopausal status (p=0.002), higher tumor stage (p=0.005) and a high proliferative index (p=0.001). A trend towards higher tumor grade, HR negativity and p53 positivity was identified. Women with BRD4+/T-bet- tumors had a significantly poorer disease free survival (p=0.0165) compared to all other combinations.
Conclusions: BRD4 expression is common in BC and is associated with adverse prognostic parameters and outcome. Its interplay with T-bet+ TILs suggests a link between the immune microenvironment and BRD4-associated tumor progression, particularly in basal cancers. This requires further exploration and may provide insight into the continued development of BRD4 inhibitors and other immune therapies.

281 The Immune Regulator Galectin 3 Is Expressed on Primary Breast Carcinomas and Their Tumor Infiltrating Lympocytes: Implications for Immune-Based Therapy

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Background: The host immune response is central to breast cancer growth, progression and response to therapy. Galectin-3 (Gal3), a lectin with diverse roles in regulating immune responses in the tumor microenvironment (TME) via expression on both immune cells and tumor cells, has been proposed as a target for immunotherapy in breast carcinoma. We evaluate labeling patterns of Gal3 on tumor cells and tumor infiltrating lymphocytes (TIL) in invasive ductal carcinomas (IDC) with known estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2) and programmed death ligand-1 (PD-L1) status to characterize expression in the pre-treatment TME and determine associations with checkpoint inhibitor expression.
Design: Gal3 IHC was performed on whole sections of 44 surgically resected primary breast IDC without prior therapy (15 HER2+ (ER-/PR-/HER2+), 14 Luminal A (LumA; ER+/PR+/HER2-), 15 basal-like (BLC; ER-/PR-/HER2- and CK5/6+ or EGFR+)); of these, 9 were PD-L1+ (>5% membranous labeling). TIL density was previously scored as none (0), mild (1), moderate (2) or diffuse (3) (<5%, 5-50%, >50% of tumor stromal area). PD-L1 expression on TIL was previously scored as none (0), focal (1+), moderate (2+), or diffuse (3+) (5%, 5-50% or 51-100%). Any strength cytoplasmic Gal3 labeling in tumor cells or TIL was scored as Gal3+. Clinicopathologic data were recorded.
Results: Gal3 tumor labeling was seen in 56% of cases (67% HER2+, 64% LumA and 47% BLC). Gal3 TIL labeling was present in 56% of cases (67% HER2+, 64% LumA and 40% BLC). Gal3+ carcinomas were more likely to have Gal3+ TIL (72% vs 22%, p=0.002). IDC with Gal3 labeling on both tumor and TIL were smaller (avg 2.2 cm vs 4.4 cm, p=0.009), and less likely to develop metastases/death (0% vs 31% p=0.02) than tumors negative in both compartments. IDC with Gal3 labeling on both tumor and TIL were also enriched for HER2+ (48%) compared to LumA (26%) or BLC (26%) (p=0.08). There was no association between Gal3 and PD-L1 tumor labeling or TIL intensity; however, diffuse PD-L1+ TIL was seen only in IDC with Gal3 labeling on both tumors and TIL.
Conclusions: Gal3 labeling is present on both IDC and TIL with enrichment in HER2+ tumors. While conclusions are limited due to small numbers, Gal3 expression on both tumor and TIL is associated with smaller tumor size, fewer metastases/deaths, and high PD-L1 expression on TIL. These findings suggest a role for Gal3 in the immune TME of IDC and support further exploration of Gal3 in IDC and its utility as a potential immunotherapeutic target.

282 Invasive Lobular Carcinomas with PTEN Loss Have Increased PD-L1+ Tumor Infiltrating Lymphocytes

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Background: The host immune response plays an important role in breast cancer progression and response to traditional and immune-based therapies. Most research to date has focused on invasive ductal carcinoma (IDC), while invasive lobular carcinoma (ILC) remains underexplored. We previously showed that, in contrast to tumor immune microenvironment (TiME) associations in IDC, ILC carcinoma cell PD-L1 expression is independent of the degree of tumor infiltrating lymphocytes (TIL), tumor grade or estrogen receptor (ER) status. This suggests that other factors may influence PD-L1 expression in ILC. Mutations in the tumor suppressor gene *PTEN* are enriched in ILC, and PTEN loss is associated with increased PD-L1 expression in multiple tumor types including triple negative IDC. We hypothesized that PTEN loss may also contribute to the PD-L1 expression patterns in ILC
Design: The TiME including the TIL composition and density and PD-L1 labeling pattern of 47 ILC was previously characterized. TMAs containing the same 47 ILC

were labeled for PTEN by IHC as previously validated in prostate cancer. PTEN loss was defined as entirely negative nuclear labeling across >10% of cells. Cases were scored as having ambiguous or equivocal PTEN expression if the tumor cell labeling was faint but not clearly lost.

Results: The ILC cohort consisted of 70% luminal A, 17% luminal B, 11% triple negative (TNBC), and 2% HER-2-only. PTEN labeling was intact in 39 (83%) cases, lost in 13% (6) cases, and equivocal in 2 (4%) cases. PTEN loss was more common in TNBC (40%) than other subtypes (88%) (p=0.08). ILC with PTEN loss were more likely to be PD-L1+ (50% vs. 13%, p=0.059) and contain high numbers of PD-L1+ TIL (>50% TIL with PD-L1 labeling; 83% vs. 23%, p=0.008). There was no association between PTEN status and TIL density or individual TIL phenotype counts.More patients with PTEN loss tended to present at stage II-III (100% vs. 59%, p=0.075) and die due to disease (33% vs. 5%, p=0.087) than did those with retained PTEN labeling.

Conclusions: A subset of ILC contains an active TiME, and PTEN loss may be one mechanism regulating PD-L1 expression in these tumors. These findings suggest PTEN status may predict tumors likely to upregulate PD-L1, and support exploring combinatorial therapies targeting both the PD-1/PD-L1 pathway and the PI3K pathway in PTEN-deficient ILC.

283 CD206 Positive Macrophages in Axillary Lymph Nodes of Patients with Breast Adenocarcinoma Express PD-L1

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Background: Macrophages are heterogeneous mononuclear cells with a high degree of plasticity. Studies in pancreas, colon, liver, and lung cancers have shown that tumor associated macrophages (TAM) with the M2 phenotype are associated with tumor metastasis, while M1 TAMs are associated with longer survival. M2 macrophages are CD206 positive and when an injury to a tissue persists, M2 macrophages play a pro-fibrotic role by recruiting fibroblasts to the site of injury. Programmed death 1 (PD-1) protein is a cell membrane receptor that is expressed by T-cells, natural killer cells and B-cells. Once this receptor is bound to its ligands (PD-L1 and PD-L2), activation of T-cells is inhibited and their regulatory function is enhanced, resulting in a decreased immune response. We determined the subtype of the macrophages in the axillary lymph nodes of patients with breast ductal adenocarcinoma and whether these macrophages express PD-L1.

Design: 15 patients with breast ductal adenocarcinoma and axillary lymph node dissection were included in this study. Twelve of these patients had at least one lymph node positive for metastatic carcinoma and the remaining 3 had no evidence of lymph node metastasis. We prepared a tissue microarray (TMA) slide from these lymph nodes and stained them for CD2016 and PD-L1. Trichrome special stain was used to assess collagen fibrosis in the lymph nodes.

Results: All 12 lymph nodes with metastatic carcinoma contained CD206 positive macrophages located mainly in the sinuses and absent where tumor cells effaced lymph nodes. In 11 of these cases, PD-L1 was co-expressed by the CD206-positive macrophages. Interestingly, macrophages within the sinuses in the 3 cases with no evidence of lymph node metastasis were double positive for CD206 and PD-L1. Collagen fibrosis was more prominent in the areas where tumor cells effaced the lymph node architecture.

Conclusions: CD206-positive macrophages expressing PD-L1 in the lymph nodes of patients with breast carcinoma suggests an environment that induces T-cell dysfunction and anergy. The presence of M2 macrophages in the sinuses not mixed with tumor cells, suggests that they are recruited by the tumor cells to pave the way for tumor invasion and metastasis. The collagen fibrosis associated with tumor cells supports pro-fibrotic effect of M2 macrophages in lymph nodes positive for metastatic carcinoma. These data support the rationale for immunotherapy to boost the immune system of individuals with breast carcinoma.

284 Histopathologic Correlates of Non-Mass Enhancement Detected by Breast MRI

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Background: Non-mass enhancement (NME) is commonly seen on dynamic contrast-enhanced magnetic resonance imaging (MRI) of the breast performed for screening and diagnostic purposes. However, the pathologic correlates of NME have not been extensively explored, and consequently it is not uncommon to be uncertain about concordance between MRI and pathologic findings. This uncertainty may cause delay in management recommendations and may compromise patient care. Thus, the focus of our study was to investigate the histologic alterations corresponding to NME.

Design: A two-year (9/1/2014-9/1/2016) retrospective analysis was performed to identify cases of NME resulting in biopsy. NME identified in patients with a known adjacent malignancy were excluded. The corresponding slides for all cases were reviewed; the pathologic alteration regarded as the dominant finding and secondary pathologic changes were documented. The dominant lesion was defined as the most prevalent alteration, not necessarily the most severe alteration.

Results: 89 patients with 93 NME lesions underwent core needle (n=90) or excisional (n=3) biopsy. A dominant lesion was identified in 93.5% (87/93); 95.7% (89/93) demonstrated multiple histologic alterations. The most frequently identified dominant lesion was DCIS (24 cases, 25.8%; 5 low, 11 intermediate, 9 high grade). Invasive carcinoma was the dominant lesion in 4 cases (4.3%; 3 IDC, 1 ILC). Among the cases in which neither DCIS nor invasive cancer was identified, LCIS was present in 1 (1.1%) and atypical lesions including ADH, FEA, and ALH were found in 8 (8.6%), 7 (7.5%), and 3 (3.2%) cases (dominant lesion in 4.3%, 0%, and 0%), respectively; 22 cases had at least focal atypia (23.7%). The most frequent benign entities included cysts (64.5%, 60/93), UDH (54.8%, 51/93), apocrine metaplasia (38.7%, 36/93), columnar cell change/hyperplasia (34.4%, 32/93), PASH (33.3%, 14/93), and apocrine cysts (22.6%, 21/93). Among benign entities, only PASH was found as the dominant lesion in an appreciable number of cases (11.8%, 11/93).

Conclusions: In this study, the dominant NME-associated lesion was DCIS or invasive cancer in 30.1%, with LCIS in 1.1% and atypical lesions in 23.7% of cases. In the remaining 45% of cases, the dominant lesion was benign. These results indicate that NME can be associated with benign, atypical, and malignant pathology. Additional studies are required to compare specific imaging findings with pathology to further improve radiology-pathology correlation in cases of NME.

285 The 21-Gene Recurrence Score in Special Histologic Subtypes of Breast Cancer with Favorable Prognosis

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Background: The 21-gene recurrence score (RS) predicts the likelihood of distant recurrence and chemotherapy benefit in early stage, ER+/HER2- breast cancer (BC). There is limited data on the RS of histologic subtypes with favorable prognosis.

Design: At our center, all lymph node negative and selected node positive ER+/HER2- BC ≥5 mm are sent for RS assay. We reviewed cases with favorable histology and available RS treated in 2006-2015. Two pathologists confirmed the diagnosis. Statistical analysis was performed using SPSS 24.0.

Results: Our cohort includes 8 tubular (TC), 5 solid papillary (SPC), 9 encapsulated papillary (EPC) and 33 pure mucinous (MC) carcinomas. One EPC and 2 SPCs had associated stromal invasion <1.5 mm. Most (44/55; 80%) cases had RS<18; none had RS≥31. RS≥18 was associated with low PR expression (p=0.002). No morphologic feature (tumor grade, biopsy site changes, cellular stroma, inflammation) was associated with RS≥18. Overall, 53 (96%) patients received adjuvant endocrine therapy and 7 (13%) chemotherapy. At median follow-up of 41 months, the distant recurrence free survival was 100%.

Table 1. Clinicopathologic characteristics of 55 cases with favorable histology

	TC (n=8)		SPC (n=5)		EP (n=9)	MC (n=33)	
RS	<18(n=6)	18-30 (n=2)	<18 (n=3)	18-30 (n=2)	<18 (n=9)	18 (n=26)	18-30 (n=7)
RS, median (range)	13 (9-14)	20 (20-20)	5 (3-7)	22 (19-25)	3 (0-11)	11 (3-17)	22 (18-28)
Age, median (range), year	47 (44-54)	58 (46-69)	59 (45-65)	70 (65-75)	64 (47-84)	59 (36-77)	52 (39-69)
Tumor size, median (range), cm	0.8 (0.6-1.8)	1.3 (1.2-1.4)	1.3 (0.7-1.4)	1.4 (1.3-1.5)	1.5 (0.6-2.4)	1.3 (0.5-3.5)	1.2 (0.5-1.6)
Grade							
1	6 (100%)	2 (100%)	0	0	2 (22%)	3 (12%)	2 (29%)
2	0	0	3 (100%)	2 (100%)	7 (78%)	23 (88%)	5 (71%)
Lymph node positive	0	0	0	0	1 (11%)	3 (12%)	0
ER%, median (range)	95 (90-99)	95 (90-100)	98 (90-99)	98.5 (98-99)	99 (90-100)	95 (20-100)	99 (70-100)
PR%, median (range)	70 (5-99)	55 (20-90)	90 (70-90)	35 (5-65)	95 (80-100)	90 (0-99)	85 (0-99)
Endocrine therapy	6 (100%)	2 (100%)	3 (100%)	2 (100%)	7 (78%)	26 (100%)	7 (100%)
Chemotherapy	0	1 (50%)	0	0	0	3 (12%)*	3 (43%)

*Multifocal, largest 3.5 cm (n=1); RS 17 (n=1); lymph node micrometastasis (n=1).

Conclusions: This is the largest series reporting the RS of BC with favorable histology. All EPCs had RS<18. But RS 18-30 was observed in other favorable subtypes, the significance of which remains unclear and warrants larger studies.

286 Breast Carcinoma with Recurrence Score Lower Than 18: Rate of Locoregional Recurrence in a Large Series with Clinical Follow-Up

Gulisa Turashvili, Edi Brogi, Monica Morrow, Alicja Wiszowaty, Maura Dickler, Larry Norton, Clifford Hudis, Hannah Y Wen. Memorial Sloan Kettering Cancer Center, New York, NY.

Background: The 21-gene recurrence score (RS) determines the benefit of chemotherapy and the prognosis with endocrine therapy in patients with early stage, ER+/HER2- breast cancer (BC). The RS predicts the risk of distant recurrence, but data on the association with locoregional recurrence (LRR) are limited.

Design: At our center, lymph node-negative ER+/HER2- BC ≥5 mm are routinely sent for RS assay, with rare exceptions. We collected data on clinicopathological features, treatment and outcome of 1405 consecutive patients with node-negative ER+/HER2- BC of RS <18, treated in 2008-2013. Statistical analysis was performed using SPSS 24.0.

Results: The patient median age was 56 years (range 22-90). The median tumor size was 1.2 cm (range 0.3-5.8 cm). At median follow-up of 52 months, 13/1405 (1%) patients developed LRR. All cases with LRR had negative margins. Sites of LRR included the ipsilateral breast (n=8), chest wall (n=3), axillary node (n=1) and internal mammary node (n=1). LRR was associated with multifocality (p=0.03), but it was not associated with age, tumor size, lymphovascular invasion (LVI) or type of local treatment. The

LRR rates of BCs with RS 0-10 and 11-17 were similar. The rate of distant recurrence was 0.4%, as we reported recently. None of the patients with LRR developed distant recurrence. The rate of LRR in patients treated with adjuvant endocrine therapy alone was 0.7% (8/1192). An exploratory analysis in this treatment group showed a 5% (2/40) absolute rate of LRR among patients aged <40 years versus 0.5% (6/1152) among patients aged ≥40 years.

Table 1. Clinicopathologic characteristics of patients treated with endocrine therapy and no chemotherapy

	Patients without LRR (n=1184)	Patients with LRR (n=8)
RS		
Median (range)	12 (0-17)	13 (0-17)
RS 0-10, n	464	3
RS 11-17, n	720	5
Age, years		
<40 years	38	2
40-50	253	1
>50 years	893	5
Tumor size, median (range), cm	1.1 (0.3-5.5)	1.8 (0.7-3.5)
LVI, n	212	3
Local treatment, n		
Breast conserving surgery (BCS) alone	39	1
Total mastectomy (TM)	333	2
BCS + radiation	810	5
TM + radiation	2	0
Time to LRR, median (range), months	-	37 (10-74)
Follow-up time, median (range), months	51 (0.8-93)	65 (26-84)

Conclusions: Patients with BC of low RS treated with only adjuvant endocrine therapy had a LRR of 0.7%, but patients <40 years old at diagnosis had a higher rate of LRR. This finding warrants further studies.

287 The University of Rochester Modified Magee Algorithm (RUMMA) for Risk Stratification of Estrogen Receptor Positive Breast Cancer Patients; a Multi-Institutional Validation Study

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Background: We published an algorithm [Turner et al. Mod Pathol. 2015] using the Modified Magee Recurrence Score (MMRS), based on risk models developed at Magee Women's Hospital [Klein et al. Mod Pathol. 2013]. Our algorithm was shown to provide similar risk information to the Oncotype DX(Genomic Health, Redwood City, CA, USA), a multigene quantitative reverse transcription-polymerase chain reaction-based assay that estimates risk of distant breast cancer (BC) recurrence and predicts treatment benefit for patients with estrogen receptor (ER) positive BC. We previously presented a single institution validation study of this algorithm (now called the University of Rochester Modified Magee Algorithm [RUMMA]). We now present a multi-institutional validation of the RUMMA, with additional new case data.

Design: We identified 395 consecutive cases with an ODX recurrence score (ODXRS), from the pathology files at the University of Rochester Medical Center and the University of Louisville Health Sciences Center. We correlated the RUMMA risk stratification criteria with the ODXRS risk stratification categories (see Table).

RUMMA criteria	ODXRS category		
	HIGH (>30)	INTERMEDIATE (18-30)	LOW (<18)
MMRS ≤ 12 (low risk)	0	4	39
NS<6, ER/PR≥150,Ki-67<10% (low risk)	0	2	29
MMRS > 30 (high risk)	14	0	0

Results: 92% of cases predicted to be low risk by the RUMMA (68/74) had a low risk ODXRS. 100% of cases predicted to be high risk by the RUMMA (14/14) had a high risk ODXRS. Of the 327 cases with a predicted intermediate RUMMA risk score, 8% had a high risk ODXRS, 38% had an intermediate ODXRS, and 54% had a low risk ODXRS. 100% of cases with a high risk ODXRS (n=41) and 97.4% of cases with an ODXRS > 25 (n = 77) had a MMRS > 15. 98.6% of cases with a MMRS ≤ 15 (n = 138) had an ODXRS of ≤ 25 (mean ODXRS = 11.4). 87% of cases with a MMRS ≤ 15 had a low risk ODXRS.

Conclusions: Our results now include data from more than one institution and continue to support the RUMMA as an alternative to ODX testing in certain ER+ BC patients. Cases in the RUMMA intermediate risk group should be considered for ODX testing, particularly if the MMRS is between 15 and 30. We hope to expand our study population to additional institutions, and evaluate outcomes, in order to further qualify the clinical utility of the RUMMA.

288 Correlation of Clinical and Pathologic Features in Pregnancy-Associated Breast Cancer

Julianne M Ubago, Mansooreh Eghtesad, Jennifer Pincus, Kalliopi P Siziopikou, Luis Z Blanco. Northwestern University Feinberg School of Medicine, Chicago, IL.

Background: Pregnancy-associated breast carcinoma (PABC) is carcinoma diagnosed during gestation or within 5 years postpartum and is associated with a poor prognosis, which is worse when diagnosed within 2 years of parturition. While previous studies have shown a unique set of pathologic features related to this subset of tumors, including higher tumor grade and associated inflammatory response, few have looked at the associated clinical aspects of these tumors. In this study, we analyzed clinical findings and correlated them with pathologic features to determine the impact that clinical factors have on PABC prognosis.

Design: Twenty-three patients with PABC diagnosed within 2 years of pregnancy (mean age=35.8 years, range=26-48) and 14 control age-/stage-matched nulliparous women (mean age 37.5 years, range=29-51) were evaluated. Slides were previously reviewed and pathologic and immunohistologic tumor characteristics were recorded. The electronic medical records were then reviewed and clinical features including BRCA status, history of infertility treatments, additional biopsies and local/regional or distant recurrence were recorded. The average follow-up was 83 months (range=70-106 months).

Results: PABC patients had a higher rate of BRCA-positivity (4/23, 17%) and infertility treatments (6/23, 26%) compared to matched controls (table 1). Interestingly, of the 6 PABC patients with infertility history, 2/6 (33%) had low grade (grade 1) tumors, while none of the other PABC patients presented with low grade tumors (0/17, 0%) (p=0.059). PABC patients also had higher rates of additional biopsies on follow-up and higher rates of tumor recurrence. No significant correlation was observed between clinical features and lymphovascular space invasion, lymph node positivity, or hormone receptor/HER2 status.

	BRCA Positive	Infertility Treatment	Additional biopsies	Recurrence Status
PABC Patients	4/23 (17%)	6/23 (26%)	10/23 (43%)	3/23 (13%)
Control Patients	1/14(7%)	1/14 (7%)	4/14 (29%)	0/14 (0%)

Conclusions: PABC patients had higher rates of all clinical features assessed and had a worse prognosis on follow-up. Yet, PABC patients who underwent infertility treatment were more likely to have low grade tumors. Our findings suggest that aspects of the patient's clinical history, as well as pathologic and immunohistologic characteristics, may influence the overall prognosis for PABC patients.

289 PD-1 and PD-L1 Expression in HER2-Positive Breast Carcinomas

Julianne M Ubago, Alexandra Larson, Mansooreh Eghtesad, Luis Z Blanco, Jennifer Pincus, Kalliopi P Siziopikou. Northwestern University Feinberg School of Medicine, Chicago, IL.

Background: 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 within this group, a subset of these tumors develop resistance urgently requiring novel therapeutic options. In addition to the classic HER2+ subtype, Luminal B tumors are another subtype of HER2-positive breast carcinomas which could benefit from new therapies. Targeting the PD-1 and PD-L1 axis may represent a potential new therapeutic strategy in breast carcinoma, especially in aggressive tumor subtypes. In this study, we evaluated expression of PD-1 and PD-L1 in both the tumor cells and tumor-infiltrating lymphocytes (TILs) in classic HER2+ and in Luminal B subsets of HER2-positive breast carcinomas.

Design: The study population consisted of 114 patients with HER2-positive invasive breast carcinoma diagnosed from 2009-2014 (mean age:53, range 18-80). Slides with tumor from each case were stained with PD-1 and PD-L1. The extent of staining (0%=0, 1-10%=1, 11-50%=2, and 51-100%=3) and staining intensity (0, 1+, 2+, 3+) were assessed in both the tumor cells and TILs. Then a composite score (CS) was calculated by multiplying extent and intensity results (range 0-9; 0-3=negative, 4-9=positive).

Results: Overall, PD-1 was positive in TILs in 57/114 cases (50%). 28/54 (52%) of TILs in HER2+ tumors and 29/60 (48%) in Luminal B tumors expressed PD-1. None of the cases examined expressed PD-1 in the tumor cells. As a group, PD-L1 was positive in the tumor cells in 10/114 (8.8%); of those, 6 were HER2+ cases (6/54, 11%) and 4 were Luminal B cases (4/60, 6.7%). Of interest, PD-L1 was expressed in 37/114 (32.4%) of the TILs. Notably, TILs expressed PD-L1 in 23/54 cases (43%) in the HER2+ subgroup while only 14/60 (23%) expressed PD-L1 in the Luminal B subgroup (p= 0.04). Finally, PD-L1 expression in TILs was more common in grade 3 tumors (30/83, 36%) than in grade 2 tumors (7/31, 23%).

Conclusions: 1. PD-L1 is expressed in TILs in almost half of HER2+ tumors and a quarter of Luminal B tumors. **2.** PD-L1 is only rarely seen in the tumor cells in these two HER2-positive subtypes. **3.** PD-1 is expressed in 50% of the TILs but is not present in the tumor cells of any of our cases. **4.** PD-L1 in TILs is more common in grade 3 carcinomas. Our findings add to the understanding of the role of host/tumor microenvironment in the classic HER2+ and Luminal B subtypes of breast tumors and raise the possibility that targeted immune-based therapeutic strategies may show a benefit against these aggressive breast carcinomas.

290 OncotypeDx and Prosigna in Breast Cancer Patients with a Predominantly Intermediate Recurrence Score: A Comparison Validation Study

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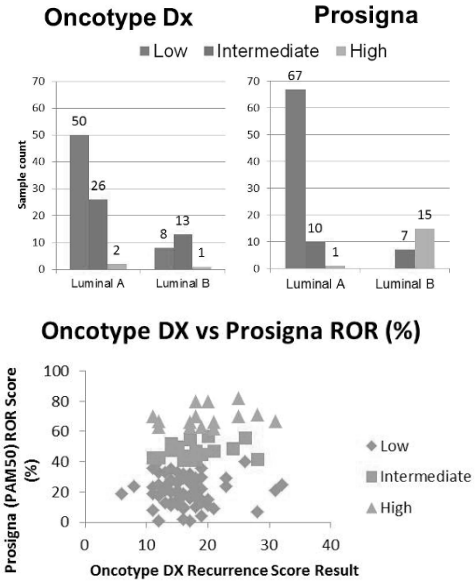
Background: Oncotype Dx® (ODX) is the most widely used prognostic and predictive assay for ER + cancers and categorized into low (< 18), intermediate (8 to 30) or high (≥31) risk recurrence score in 10 yrs. Prosigna® is another prognostic signature to estimate distant recurrence-free survival for stage I/II, ER+ cancer in postmenopausal women treated with adjuvant therapy. The goal of the study is to assess the agreement between ODX and Prosigna® for patients that were previously classified by ODX.

Design: A cross sectional study using a random sampling of 100 previously ODX classified peri and post-menopausal patients, stage (I or II) breast cancer from the pathology database were retrieved. We performed RNA isolation according to standard protocols provided by NanoString, and ran the assays on a NanoString nCounter® DX Analysis System. Descriptive analyses of clinical, pathological, and genomic factors on the different Prosigna-classified groups were performed.

Results: The average age was 62.4 year (range 43 to 85). 20% of the patients were perimenopausal and 80% postmenopausal. Cancers include invasive ductal (78%), invasive lobular (18.0%), and mixed carcinoma (4.0%); Nottingham grades encompass grade 1 (36%), grade 2 (55%) and grade 3 (9%). 63% had lumpectomy and 37% underwent mastectomy. 9.0% had positive lymph nodes. Treatment includes radiation (57%); chemotherapy (28%), and hormonal therapy (96%). ODX assay were assigned as follows: 58% low, 3% high and 39% intermediate. There were 8% two-step disagreements (high to low or vice versa) between ODX and Prosigna®; and 42% one-step disagreement (low to intermediate or intermediate to high) (spearman's correlation = 27.8%).



Conclusions: Some patients are classified into different risk categories when using different genomic platforms (ODX and Prosigna®), and this has management implications especially for the use of chemotherapy when needed.

291 *BRCA1* Mutations Detected by Next-Generation Sequencing in Sporadic Breast Cancer with Medullary Histological Features Correlate with Hereditary Disease

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Background: The role of somatic *BRCA1/2* gene mutations in breast cancer is getting increasing attention in view of a hereditary disease. No strict histological criteria exist for the selection of patients undergoing *BRCA1/2* germline mutation testing in clinically sporadic patients. The medullary phenotype and triple negative intrinsic subtypes are often but not exclusively encountered in *BRCA1* germline mutated breast cancer, for *BRCA2* no association to specific histological features are known. In this study, we analysed if any relationship exists between medullary phenotype and *BRCA1/2* somatic mutations in terms of hereditary breast cancer.

Design: 32 clinically sporadic breast cancer cases with medullary features suggesting hereditary origin were analyzed for somatic *BRCA1/2* mutations (all coding exons) with next-generation sequencing (NGS) technology. Paraffin embedded formalin fixed breast cancer samples from all patients and normal breast tissue from one patient were analyzed

Results: Three of 32 cases had definitely pathogenic changes in the *BRCA1* gene. Two of them had deletions leading to frameshift mutations (p.Glu23fs, p.Val1234fs), one SNV lead to a premature STOP codon (p.Glu60Ter) in the tumor tissue (9.38 %). In one patient normal breast tissue was available where the same pathogenic *BRCA1* mutation was detected (p.Glu23fs) as in the corresponding tumor tissue. Retrospective follow-up in the other two patients revealed family history of breast cancer and recent records on germline *BRCA1* mutation testing which confirmed our findings. Non-pathogenic alterations according to the ARUP and ClinVar databases were detected in five patients. Additionally, one previously unreported mutation in the *BRCA1* gene was identified (p.Leu2654Val) which is predicted to alter the protein function using bioinformatics tools.

Conclusions: Definitely pathogenic *BRCA1* mutations in clinically sporadic breast cancer with medullary features were in retrospect associated with positive family history and/ or *BRCA1* germline mutations. Somatic *BRCA1/2* mutation-assays can be tested on paraffin embedded tumor tissues within the routine diagnostic service. In case of proven pathogenic *BRCA1* mutations without known positive family history of breast cancer this testing can deliver clinically relevant information in terms of genetic counseling, though further confirmation of these data remains necessary.

292 Intra-Tumoral Injection of Acetic Acid and Ethanol Causes Tumor Regression of Human Breast Cancer in Xenograft Murine Model

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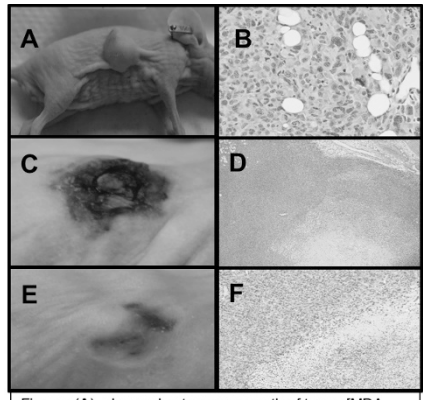
Background: The current trend in breast cancer treatment is to decrease surgery and be more conscientious of cosmetic results. Neoadjuvant chemotherapy allows patients the opportunity to reduce tumor size prior to surgical procedures in hopes of a less aggressive surgery. Intratumoral injections using chemotherapeutic agents have been tried to aid in tumor regression. However, no trials with simple chemicals with no known systemic side effects have been attempted. We evaluated local oncotoxic effects of ethanol and acetic acid in a breast cancer xenograft murine model.

Design: Breast cancer cell line MDA-MB-231 was used to grow subcutaneous tumors in 30 SCID mice, subdivided as follows: 10 acetic acid, 10 ethanol and 10 saline controls. Once the tumor size reached > 62.5 mm^3 , the tumors were injected with either, 25% acetic acid, 100% ethanol or saline. Post treatment, one mouse per group was euthanized after 2 hours, 24 hours, 7 days, and 14 days for histological analysis. The remaining 6 mice/group were followed for 120 days. Mice were euthanized after they met the humane criteria for tumor burden and overall health or at 120 days, post treatment.

Results: Most mice implanted with tumor cells showed tumor regression post injection of acetic acid by 24 hours (n=7/9). Histological examination showed tumor degeneration and extensive necrosis with blood vessel obstruction. This same reaction was noted in only 2 of 9 animals in the ethanol-treated group and in none of the saline treated group. By 1-2 weeks post injection, there was a decrease in response, resulting in complete tumor regression in only 4/6 mice in both the acetic acid and ethanol-treated group by 120 days.



Figures (**A**)- show subcutaneous growth of tumor [MDA-MB-231 cell lines]. (**C&E**)- show ulceration after injecting acetic acid and ethanol respectively, (**B**,**D&F**)- H&E; show extensive necrosis after injecting normal saline, acetic acid and ethanol respectively.

Conclusions: In this xenograft murine model, direct injection of acetic acid, and to a lesser extent ethanol, caused tumor regression, and sometimes even eradication. This could potentially be used in humans as an adjunct or instead of neoadjuvant chemotherapy to reduce tumor size and aid in a more cosmetically appealing result. Further plans include studying additional dosing's and concentrations of cytotoxic agents in order to produce better results without any significant side effects.

293 IgG4-Related Sclerosing Disease of the Breast in a Male Patient

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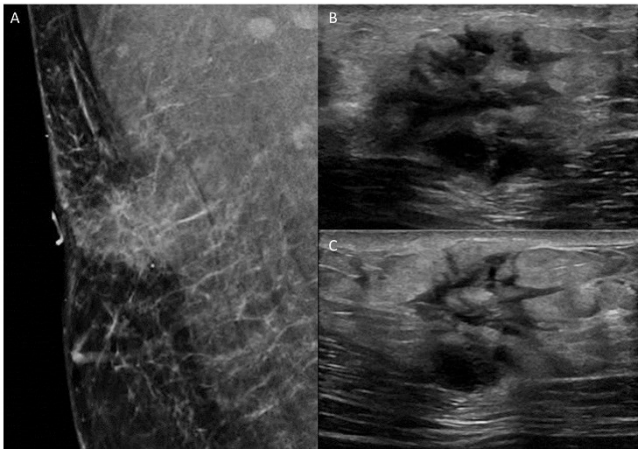
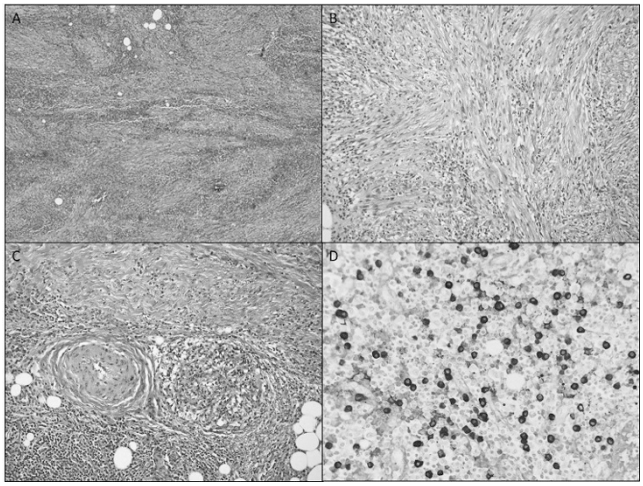
Background: IgG4-related sclerosing disease of the breast is a rare entity. We report the first case in a male patient. A 48-year-old male presented with 4-week history of palpable right upper outer quadrant breast mass associated with skin puckering. Past medical history included hypertension. Family history included breast cancer in his aunt at age 55. Mammography (Figure 1A) and ultrasound (Figure 1B,C) showed a suspicious spiculated 2.5 cm mass with skin retraction and extension to the pectoralis muscle. Ultrasound-guided core biopsy was performed.



Design: Core biopsy and resected tissue was submitted for pathologic examination. Formalin-fixed, paraffin embedded tissue was prepared for H&E and IHC, including CD3, CD5, CD20, CD30, CD10, BCL-6, Ki-67, BCL-2, CD21, CD43, PAX-5, CD138, ALK-1, AE1.3, CD163, IgG and IgG4. In situ hybridization for kappa, lambda, and EBER were performed.

Results: Core biopsy was interpreted as acute and chronic inflammatory process. No ductular breast tissue was present. IHC showed a mixed population of B-cells and T-cells and polytypic plasma cells. Discordance with imaging findings prompted surgical excision. Sections showed ill-defined yellow-brown fatty tissue resembling fat necrosis. Histology showed fibrosis, dense lymphoplasmacytic inflammation (Figure 2A,B), and focal obliterative phlebitis (Figure 2C). IgG and IgG4 showed IgG4-positive plasma cells with counts of over 50 per HPF (Figure 2D). Subsequent workup showed no other organ involvement and serum IgG4 53.8 mg/dL (normal range 2.4-121 mg/dL).



Conclusions: It is important for pathologists to include this entity in the differential diagnosis of a breast mass in male and female patients as these lesions have shown good response to steroid therapy, avoiding unnecessary surgery.

294 Abnormal Loss of c-kit Is Associated with Malignant Transformation in Mammary Epithelium and May Be a Consequence of KIT Promoter Methylation

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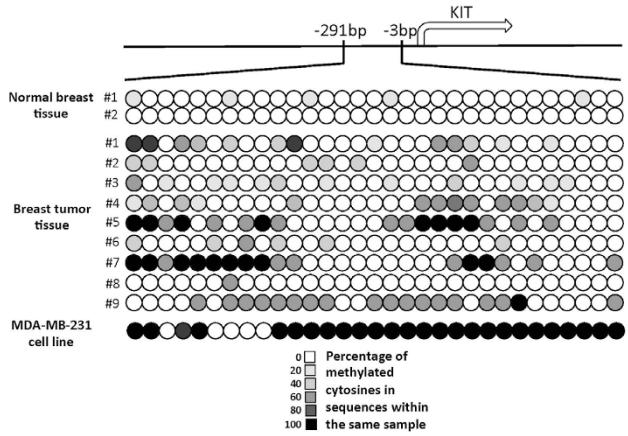
Background: C-kit is a transmembrane tyrosine kinase that is known to be lost often during malignant transformation of the mammary epithelial cells. The mechanism of this c-kit down regulation is unknown. Studies of whole exome sequencing of breast carcinomas have failed to demonstrate significant mutations of the KIT gene, indicating alternative pathways involved. We hypothesize that c-kit down regulation in breast carcinomas may be a result of c-kit promoter methylation.

Design: 161 cases of invasive mammary carcinomas (including ductal, lobular and triple negative subtypes) were stained for c-kit and normal adjacent breast tissue was used as internal control. All 161 cases showed loss of c-kit expression in the tumor, which was preserved in normal mammary epithelium. Samples from 9 tumor, 2 normal breast parenchyma and 1 from breast carcinoma cell line (MDA-MB-231) were used for for molecular studies. Following DNA extraction from tumor and normal breast, purified DNA was subjected to bisulfide conversion and the region corresponding to c-kit promoter encompassing 300bp upstream of transcription initiation site was sequenced. The sequences of promoter regions from tumor were compared to normal breast and the differences in promoter methylation were determined.

Results: We identified CpG islands within the c-kit promoter immediately upstream of transcription initiation site. Bisulfide sequencing analysis revealed that cytosines in CpG dinucleotides within c-kit promoter region of tumors were highly methylated compared to normal breast. There was heterogeneity in tumors, which is explained by imperfect separation of tumor mass from adjacent non-tumor tissue. To further support the hypothesis we analyzed c-kit promoter methylation of aggressive triple negative breast cancer cell line (MDA-MB-231) and found that 95% of cytosines in CpG dinucleotides within sequenced region were also methylated.



Conclusions: Methylation of cytosines in the KIT promotor region was increased in the breast carcinoma cells as compared to normal breast epithelial cells. We believe that the loss of c-kit expression in malignant breast lesions may be associated with epigenetic silencing of c-kit gene through methylation of CpG islands in its promoter region.

295 Discordant HER2 Immunohistochemical Expression and Gene Amplification by In-Situ Hybridization in Ductal Carcinoma In Situ

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Background: Ductal carcinoma in situ (DCIS) is recognized as a precursor to invasive ductal carcinoma (IDC). HER2 overexpression by immunohistochemistry (IHC) is more frequently observed in DCIS (40-60%) in comparison to IDCs (25%). The molecular mechanism is presently unclear. However, some reports suggest that HER2 overexpression by IHC in the DCIS component has predictive and prognostic value. Chromogenic in situ hybridization (CISH) is a method that allows for a more exact comparison between IHC expression and DNA amplification in the same microscopic focus. We aimed to assess the concordance of HER2 overexpression in DCIS and concurrent IDCs by comparing the two components using HER2 IHC and CISH.

Design: Cases of DCIS with concurrent IDC undergoing reflex HER2 CISH testing for 2+ IHC from 12/2015 to 9/2016 were reviewed. ER and PR IHC results were also collected. HER2 expression in both DCIS and IDC on CISH were scored by HER2/CEP17 ratio and average HER2 copy number based on the 2013 ASCO/CAP guidelines.

Results: Forty-four cases (n=44) meeting inclusion criteria were identified (Table 1).

	DCIS	IDC	
ER Positive	40	39	p = 0.61
ER Negative	4	5	
PR Positive	34	36	p = 0.074
PR Negative	10	8	
HER2 IHC 3+	21	0	p = 4.0 x 10-8
HER2 IHC 2+	23	44	
HER2 CISH Positive	3	2	p = 0.99
HER2 CISH Negative	41	42	

All IDCs were equivocal by IHC with 2+ staining. In contrast, 21 cases (21/44, 47.7%) showed DCIS with 3+ HER2 staining ($p<0.001$). ER and PR staining was similar between the two corresponding lesions with only 3 cases (3/44, 6.8%) showing discordant ER and PR between DCIS and IDC. Among the 21 DCIS cases with 3+ HER2, there were no significant differences in HER2/CEP17 ratio ($p=0.99$) or HER2 copy number ($p=0.083$) compared to the corresponding IDCs. Three DCIS cases (3/44, 6.8%) were positive for HER2 by CISH. One CISH-positive DCIS case (1/44, 2.3%) had corresponding IDC that was CISH-negative (ratio 2.02 for DCIS, 1.32 for IDC). In the entire cohort, there were no significant differences in CISH results based on ratio ($p=0.99$) or copy number ($p=0.83$).

Conclusions: There is a significantly greater number of DCIS with 3+ HER2 IHC staining compared to the corresponding IDCs. Paradoxically, positive HER2 IHC does not correlate with a high HER2 DNA amplification in DCIS by CISH. Our data suggests that HER2 IHC staining in DCIS overestimates true HER2 overexpression. The prognostic significance of HER2 IHC overexpression may need to be reconsidered.

296 CD4/CD8 Ratio of Tumor-Infiltrating Lymphocytes (TIL) at the Tumor-Host Interface (THI) Has Prognostic Value in Triple Negative Breast Cancer (TNBC)

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Background: Compelling evidence has demonstrated the prognostic significance of TIL in various tumor types, including BC. Further, a brisk infiltration of T cells in primary tumors has proved to be a favorable prognosticator in a number of solid cancers. There

have been only limited studies investigating the importance of subsets of T cells in TIL. Moreover, the significance of TIL at the THI vs. within the intratumoral stroma remains controversial. On the other hand, there is increasing evidence of the role of TIL in TNBC which is generally associated with a poor clinical outcome but is highly heterogeneous. This study was aimed in exploring the prognostic relevance of the subsets of TIL in TNBC.

Design: Consecutive cases of pre-chemotherapy TNBC in the authors' institution were retrieved between 2001 and 2015. Those with bilateral breast cancer, distant metastasis at diagnosis, a second malignancy or without systemic chemotherapy and those without a THI in the tissue sections were excluded. The THI was defined as within one high power field (HPF; 0.5 mm) of the invasive front. This resulted in a total of 42 cases meeting the inclusion criteria. The CD4+ and CD8+ T cell count was expressed as per HPF on average after examination of 10 HPFs.

Results: There was a wide range of CD4+ and CD8+ T cells both at the THI (26-584 and 23-410, respectively) and within the intratumoral stroma (1-219 and 1-152, respectively). The CD4+ and CD8+ TIL were significantly higher at the former than the latter (178 vs. 31 and 143 vs. 29; both P<0.0001). The number of CD4+ or CD8+ T cells, either at the THI or within the intratumoral stroma, was not significantly associated with distant relapse-free or overall survival (RFS/OS), likely due to their wide range of distribution. However, the CD4/CD8 ratio of TIL at the THI was significantly associated with both RFS (hazard ratio [HR] 0.2, P=0.002) and OS (HR 0.13, P=0.002), while this association was not seen with the CD4/CD8 ratio of intratumoral TIL. As expected, both tumor size and nodal status were significantly associated with RFS and OS.

Conclusions: Previous studies have suggested that a specific cytotoxic T-cell response depends upon activated Th1-type CD4+ cells and that the inability of tumor rejection by the host may be due to the insufficient generation of tumor-specific CD4+ cells. Our findings further emphasize the importance of the subsets of T cells in the tumor micromilieu of TNBC. Further, TIL at the THI, but not within the intratumoral stroma, appear to be of clinical relevance.

297 GATA3 Is Negative in Endosalpingiosis: A Useful Marker in Distinguishing Metastatic Breast Carcinoma from a Benign Mimicker

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Background: Endosalpingiosis is a benign Mullerian inclusion that can mimic metastatic well differentiated breast carcinoma, particularly when encountered in axillary lymph nodes. In these challenging cases, a misdiagnosis may result in significant clinical consequences. Previous studies have suggested that use of an immunopanel consisting of Pax-8 and WT-1 can help differentiate the two entities. GATA3 has been proposed as a useful immunostain in the identification of primary and metastatic breast carcinomas, with up to 100% labeling of estrogen receptor (ER) positive breast carcinoma. However, there is limited data on the expression of GATA3 in endosalpingiosis. The aim of this study is to evaluate GATA3 labeling in endosalpingiosis to determine if GATA3 immunohistochemistry may be useful in differentiating metastatic well differentiated breast carcinoma from endosalpingiosis.

Design: Fifteen cases of documented endosalpingiosis were retrospectively identified from the pathology archives, including 2 cases of axillary lymph node endosalpingiosis and 13 cases of subdiaphragmatic endosalpingiosis. Cases classified as atypical endosalpingiosis were excluded. The 2 cases of axillary endosalpingiosis involved sentinel lymph nodes from 2 patients with primary infiltrating breast carcinoma. The 13 separate foci of subdiaphragmatic endosalpingiosis were from 11 consecutive patients and consisted of endosalpingiosis involving: pelvic lymph nodes (n=5), myometrium (n=3), paratubal soft tissue (n=2), pelvic sidewall (n=1), periadnexal soft tissue (n=1), and ovary (n=1). Whole sections of all cases were labeled by immunohistochemistry for GATA3, with any nuclear labeling considered positive. Lymphocytes served as positive internal control.

Results: All 15 cases of endosalpingiosis were negative for GATA3, all with positive internal control lymphocytes cells labeling. Strong, diffuse positive GATA3 labeling was seen in benign Walthard nests, and focal weak labeling was seen in two sections of benign fallopian tube epithelium, specifically within the ciliated and secretory cells.

Conclusions: GATA3 is a useful marker to distinguish metastatic well differentiated breast carcinoma from endosalpingiosis. The presence of focal labeling in the benign fallopian tube epithelium suggests that while focal GATA3 labeling could theoretically be present in some cases of endosalpingiosis, the patchy and weak-moderate nature of the staining would be helpful in the distinction from metastatic low grade breast carcinoma, which typically demonstrates strong and diffuse GATA3 labeling.

298 Intraoperative Assessment of Sentinel Lymph Nodes in Breast Cancer Patients Post-Neoadjuvant Therapy

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Background: Recent shift towards minimizing axillary lymph node dissection (ALND) in breast cancer (BC) patients has led to sentinel lymph node (SLN) biopsy in patients managed by neoadjuvant systemic therapy (NAT). Assessment of SLN by frozen section (FS) is used to determine the need for ALND intraoperatively, but only few studies have examined the accuracy of SLN FS after NAT. Our objective is to compare the accuracy of SLN FS in BC patients with and without NAT, and to identify features that may predict accuracy.

Design: We retrospectively identified 161 SLN FS from 49 NAT patients and 255 SLN FS from 88 non-NAT patients from 2010-2015. The FS results and permanent sections with immunohistochemistry and clinico-pathologic data were analyzed.

Results: The sensitivity, specificity and accuracy of FS analysis were comparable between NAT patients and non-NAT patients (69 vs 50%, 100 vs 100% and 90 vs 82%, respectively). In the NAT group, 5 of 49 (10%) patients had discordant results, most often due to undersampling (tumor absent on FS slide). One of these patients subsequently underwent ALND. Discordant results (all false negatives) were significantly more likely in NAT patients with ER+/HER2- status, in SLN with pN1mic and pN0i+, and with more SLN retrieved at FS (Table 1). Age, pre-NAT lymph node status, histologic type, nuclear grade, tumor size and response to NAT showed no statistical significance. For non-NAT cases large tumor size, lobular type and SLN with pN1mic and pN0i+ were associated with false negative FS assessment.

Conclusions: SLN FS diagnosis post-NAT has comparable sensitivity, specificity and accuracy to the SLN FS diagnosis in the non-NAT setting.

		NAT Cases			Non-NAT Cases		
		Concordant	Discordant	p	Concordant	Discordant	p
Avg number of SLN at FS		3.1	4.6	0.039	3.1	3.4	0.302
Radiologic tumor size	T0	0	0	0.081	0	0	0.027
	T1	4	0		46	5	
	T2	27	1		15	8	
	T3	11	4		9	2	
	NA	2	0		2	1	
Histologic type	IDC	41	4	0.359	61	11	0.022
	ILC	1	0		5	5	
	Other	2	1		6	0	
Type of LN metastasis	No metastasis	33	0	<0.001	56	0	<0.001
	ITC	0	2		0	8	
	Micrometastasis	1	2		2	6	
	Macrometastasis	10	1		14	2	
Biomarkers	ER+/HER2+	10	0	0.022	8	3	0.404
	ER+/HER2-	12	5		50	12	
	ER-/HER2+	3	0		4	0	
	ER-/HER2-	19	0		8	0	
	ER+/HER2 equiv	0	0		1	1	
	ER-/HER2 equiv	0	0		1	0	

299 Expression of GATA 3, c-KIT and GCDFP in Benign and Malignant Apocrine Lesions of the Breast

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Background: Apocrine metaplasia is a common benign finding in breast. However, it has been reported that similar molecular alterations are identified in apocrine metaplasia and invasive apocrine carcinoma (IAC), indicating apocrine metaplasia might be a precursor for malignancy. This study aimed to assess the expression of GATA 3, c-KIT and GCDFP15 expression in benign and malignant apocrine lesions.

Design: The expression of GATA 3, GCDFP15 and c-KIT were studied in twenty eight breast tissue samples by immunohistochemistry (IHC) including apocrine metaplasia (7), apocrine ductal carcinoma in situ (DCIS) (8) and IAC (13). The stains were considered to be positive for these markers if more than 20% of the cells were labeled. The benign breast tissues were used as control.

Results: Seven of seven apocrine metaplasia (100%) were positive for GCDFP15, 1 of 7 was positive for c-KIT (14%), 1 of 7 was positive for GATA3 (14%). Eight of eight apocrine DCIS expressed GATA3 (100%), 7 of 8 expressed GCDFP15 (88%), and 1 of 8 expressed c-KIT (13%). Eleven of thirteen IAC were positive for GATA3 (85%); 2 poorly differentiated IAC were negative for GATA3, 9 of 13 were positive for GCDFP15 (69%), and 0 of 13 was negative for c-KIT. Normal breast tissue showed 100% GATA3 and c-KIT positivity, and 5% GCDFP15 positivity. Staining pattern by IHC was nuclear for GATA3, cytoplasmic for GCDFP15, and cytoplasmic-membranous for c-KIT.

Table 1 GATA 3, GCDFP and c-KIT expression in apocrine lesions of breast

	Normal Breast (n=28)	Apocrine metaplasia (n=7)	DCIS (n=8)	IDC (n=13)
GATA3	100%	14%	100%	85%
GCDFP15	5%	100%	88%	69%
c-KIT	100%	14%	13%	0

Conclusions: In our study, GCDFP15 expression was associated with apocrine differentiation in benign and malignant apocrine lesions. GATA3 expression was present in majority of DCIS and IAC except in 2 poorly differentiated IAC. c-KIT expression was completely lost in all invasive apocrine carcinoma. These findings suggest that GATA3 and c-KIT could be employed as a potential diagnostic marker in evaluating breast lesions from benign to malignant apocrine lesions.

300 Do Tumor Characteristics Predict Changes in Breast Cancer Biomarkers Following Neoadjuvant Chemotherapy?

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Background: Neoadjuvant chemotherapy (NAC) prior to surgical excision is associated with decreased tumor size and improved surgical outcomes in the treatment of breast cancer in a subset of patients. Previous studies have reported changes in biomarker status following NAC; however, there has been little investigation of factors that may correlate with tumor biomarker concordance between pre- and post-NAC. Such information may help guide the selection of which NAC treated tumors to retest and thus focus healthcare expenditures to be more impactful. Our aim was to evaluate the relationship between tumor characteristics and biomarker status stability post-NAC.

Design: A retrospective search was performed to identify cases from 2012-2015 with invasive breast carcinoma diagnosed via biopsy that were subsequently treated with NAC and surgical resection. Cases in which biomarker (estrogen receptor (ER), progesterone receptor (PR), and HER2) testing was performed on both the pre-NAC biopsy and post-NAC excision specimen were reviewed to identify discordances in biomarker status (defined as a change from positive to negative or negative to positive) upon repeat testing. Tumor characteristics assessed for correlation with biomarker stability included patient age, tumor grade, tumor size at biopsy and resection, tumor triple positivity and negativity at biopsy, and node status.

Results: 83 NAC treated breast resections with residual invasive carcinoma had repeat testing of at least 1 biomarker. At biopsy, this cohort was 27% ER+/PR+/HER2-, 10% ER+/PR-/HER2-, 13% ER+/HER2+, 5% ER-/PR-/HER2+ or equivocal, 42% ER-/PR-/HER2-, and 4% other. 30% (25/83) demonstrated changes in pre-NAC biopsy vs post-NAC resection biomarker status. The rate of biomarker discordance was 8% (6/75) for ER, 18% (13/73) for PR, and 5% (4/77) for HER2, with the majority changing from positive to negative (70%). Tumors that did not demonstrate any differences in biomarker status were more likely to be triple negative prior to NAC (triple negative with no changes, 91% vs not triple negative, 58%, $p < 0.01$). There was no impact of age, grade, tumor size at biopsy or resection, tumor triple positivity on biopsy, or node status on biomarker concordance/discordance post-NAC.

Conclusions: Triple negativity on biopsy correlated with unchanged biomarker status post-NAC, but other tumor characteristics did not predict biomarker stability.

301 Immune Profiling of HER2 Positive TILs Rich Breast Cancer: Correlation with Response to Neoadjuvant Therapy

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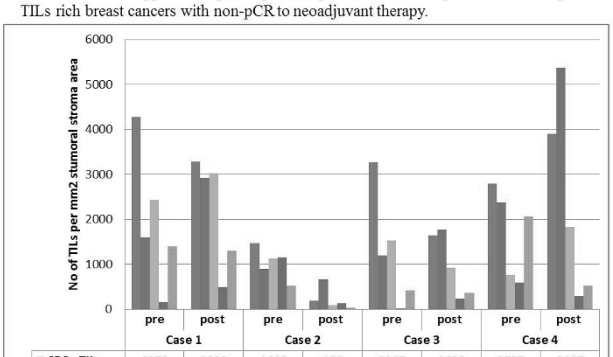
Background: Recent studies have shown that the presence and extent of tumor infiltrating lymphocytes (TILs) in tumors are both prognostic and predictive of response to therapy in HER2 positive breast cancer. The aim of the current study was to evaluate immune profiles in HER2 positive TILs rich breast cancer and correlation with response to neoadjuvant therapy.

Design: 24 HER2 positive breast cancers with TILs infiltrate greater than 30% were identified. The response to neoadjuvant therapy, pathologic complete response (pCR) or non-pCR was determined. A panel of 6 immune markers including CD3, CD4, CD8, CD68, FoxP3 and PD-L1 was assessed using immunohistochemical staining performed on pre-treatment core biopsy samples (for pCR and non-pCR pateints) and post-treatment surgical samples in the non-pCR group. Immune subsets were evaluated using the Aperio digital pathology system. H-score was used for PD-L1 evaluation.

Results: Among 24 patients, 14 (58%) experienced a pCR. There was no significant difference of any of immune subsets between the pCR and non-pCR groups. Among 10 non-pCR patients, paired pre- and post-treatment specimens were available for 4. In the 3 out of 4 paired non-pCR samples, $CD3^+$ TILs decreased in the post-treated samples compared with pre-treated samples and $CD4^+$ TILs increased in the post-treated samples after neoadjuvant therapy (Table 1). $FoxP3^+$ TILs decreased in all of 4 post-treated samples in the non-pCR group. Among 10 non pCR patients, high PD-L1 expression in malignant cells was found in the two patients, one in the pre-treatment sample and the other in the post-treatment sample. PD-L1 expression in tumoral stroma was increased highly in post-treated surgical sample compared with pre-treated biopsy sample in one out of four non pCR cases with paired pre- and post-treated samples. PD-L1 was not highly expressed in any of 14 pCR cases.

Table 1. TILs subtypes in the paired pre- and post-treatment samples in the HER2 positive TILs rich breast cancers with non-pCR to neoadjuvant therapy.



	Case 1		Case 2		Case 3		Case 4	
	pre	post	pre	post	pre	post	pre	post
CD3+ TILs	4273	3282	1463	188	3267	1639	2797	3907
CD4+ TILs	1602	2920	898	664	1200	1776	2379	5368
CD8+ TILs	2439	3030	1128	85	1530	916	769	1836
CD68+ TILs	162	493	1148	127	12	232	597	282
FOXP3 + TILs	1401	1298	518	24	424	360	2058	522

Conclusions: Immune subtypes in the breast tumor are varied in patients with different response to neoadjuvant therapy. TILs subsets in the tumor are changed after neoadjuvant. Pathological digital system is a potential tool in immune profiling analysis. To further understand immune subsets in the breast tumor with response diversity to therapy, more cases need to be evaluated for immune profiling.

302 Spectrum of Pathologic Findings in Breast Biopsy for MRI-Detected Non-Mass-Like Enhancement

Zhaohai Yang. Penn State Hershey Medical Center, Hershey, PA.

Background: With the increasing use of MRI in breast cancer screening and the biopsy of non-mass-like enhancement (NMLE), there have been several reports on the radiologic and/or pathologic characteristics of NMLE, with variable results. I sought to review the spectrum of pathologic findings in those biopsies performed at my institution.

Design: The pathology reports of breast biopsies for NMLE were retrospectively reviewed and the spectrum of pathologic findings was tabulated and correlated with the patient's clinical history.

Results: One hundred and thirteen (113) cases of breast biopsy for NMLE performed at my institution within the past 6 years were retrieved; the mean age of the patients was 52 (median 51, range 27-78). The ratio between right and left breast biopsies were 1.69. Sixty-two patients had a prior personal history of invasive breast carcinoma or DCIS; the ratio between ipsilateral and contralateral carcinomas was 1.26. Among the 113 biopsies, 74 cases (65%) had more than one pathologic finding (median 2, range 0-6). Twenty-five cases were diagnosed as carcinoma: 10 (9%) invasive carcinomas and 15 DCIS (13%). The carcinoma diagnosis on NMLE was strongly associated with a prior history of carcinoma (Fisher exact test $p=0.02$) and older age (61 vs 49 years, student's t test $p<0.0001$), but not with laterality ($p=0.3$). Six additional patients were diagnosed as ADH (5%), thus the total number of patients requiring further surgical management was 31 (27%). Seven patients (6%) were diagnosed as lobular neoplasia (7%)), flat epithelial atypia (2%), radial scar (2%) or microglandular adenosis (1%) (some patients had more than one diagnosis, as mentioned earlier), who may or may not undergo immediate surgery depending on the individual patient. The remaining 75 cases (66%) carried a benign diagnosis, including (in decreasing order) fibrocystic change (42%), usual ductal hyperplasia (36%), columnar cell change (22%), sclerosing adenosis (18%), small papilloma (13%), PASH (11%), stromal fibrosis (9%), fibroadenoma (5%), duct ectasia (5%), papillary apocrine metaplasia (4%), normal (4%), hemangioma (2%), inflammation (2%), biopsy site change (2%), and fat necrosis (1%).

Conclusions: A significant portion of patients (22%) with MRI-detected NMLE were diagnosed as invasive carcinoma or DCIS, which was associated with a personal history of breast carcinoma and older age. A total of 27% patients with NMLE required surgical management. This study further supports the value of MRI in breast cancer screening and biopsy of NMLE.

303 Novel Fluorescent Co-Localization of Myoepithelium in Breast Tissues Evaluated for HER2 by FISH

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Background: One of the drawbacks of fluorescence in situ hybridization (FISH) testing of solid tumors, including HER2 gene assessment in breast cancer is the lack of clear visualization and separation of invasive from non-invasive (benign and in-situ) foci in tissue sections evaluated by fluorsecence microscopy. While bright field ISH techniques can bypass this problem by providing a straightforward means of examining the tissue under bright light conditions, they are not an adequate alternative to FISH testing. Therefore, if a mechanism that allows for proper distinction of invasive from non-invasive areas, particularly in mixed lesions becomes available, is can render FISH interpretation exclusively on invasive tumors significantly easier and more accurate. The exclusive expression of protein antigens in myoepithelial cells makes these cells a great target to identify and isolate.

Design: We selected 15 sections of breast cancers that show significant overlap of benign changes and/or ductal carcinoma in situ and invasive tumor in the same tissue section. Following routine HER2 testing by FISH using FDA-approved protocol of IQ FISH assay (Dako/Agilent), we directly conjugated p63 mouse monoclonal antibody (clone 4A4; Biocare Medical) with Alexa Fluor mouse IgG labeling reagent (Molecular Probes) and applied it on the tissue section prior to application of DAPI. The sections were also incubated with the same p63 monoclonal antibody using routine immunohistochemistry (with polymer detection system). The H&E, IHC and FISH slides were compared for each tumor.

Results: In 15 out of 15 sections (100%), identification of benign or in-situ foci in each section was accomplished by visualization of fluorescently-labeled p63 positive myoepithelial cells. A rim of fluorescent myoepithelium was observed enabling a relatively easy separation of myoepithelium-invested nests from invasive nests. The former were excluded from HER2 gene analysis by FISH, which was reliably restricted on the invasive tumor nests.

Conclusions: This novel approach has the potential of fine tuning HER2 FISH interpretation by reliably separating invasive from non-invasive nests, particularly in tumors with different HER2 gene status between in-situ and inavsive disease. It also has potential applications beyond breast cancer, such as prostate cancer and any solid tumor FISH application involving lesions with basal cell or myoepithelial cell layer, the identification of which leads to better localization/isolation of target invasive tumro cells.

304 The Differently Expressed Genes Between Low ER Breast Cancer and Triple Negative Breast Cancer: Perspective from a Mixed Asian Ethnicity Cohort in Singapore

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Background: Low estrogen receptor (ER)-positive breast cancer is defined by expression of ER by 1% to 9% of the tumor cells seen on immunohistochemistry. A significant proportion of low-ER positive tumors have been reported to be non-luminal tumors. To identify novel diagnostic, prognostic and therapeutic targets, we investigated transcriptional profile of low ER-positive breast cancers and triple negative breast cancer (TNBC) in a Singaporean cohort representing a heterogeneous population of south-east Asian origin.

Design: 323 Samples from TNBC and 13 low ER-positive breast cancers diagnosed between 2003 and 2013 were sent for a quantitative, digital gene expression NanoString assay to measure expression of a commercial panel of 770 cancer progression-associated genes.

Results: Out of the 1199 genes panel, 38 genes are identified as differently expressed genes (DEGs). According to Ingenuity Pathway Analysis (IPA) l analysis, they are significantly overlapped with IL-15 signaling, insulin growth factor-1 (IGF-1) signaling, Pyrimidine Ribonucleotides De Novo Biosynthesis, Pyrimidine Deoxyribonucleotides De Novo Biosynthesis I , Pyrimidine Ribonucleotides interconversion and estrogen receptor signaling (table 1). Estrogen receptor alpha gene (*ESR1)* showed lower mRNA expression in TNBC compared to low ER breast cancer. However, estrogen receptor beta (*ESR2*) was not significantly different in these two groups. Mediator Complex Subunit 12(*Med12*), a known regulator of ER signaling showed higher expression in TNBC ($p<0.039$) compared to low ER-positve breast cancers.

Canonical Pathway	Genes
Estrogen receptor signaling	ESR1 MED12
IGF-1 signaling	PIK3R2 PTK2 SRF
IL-15 signaling	PIK3R2 PTK2 VCAM1
Pyrimidine ribonucleotide de novo biosynthesis	NME4
Pyrimidine deoxyribonucleotide de novo biosynthesis	NME1
Pyrimidine ribonucleotides interconversion	NME1

Conclusions: 38 DEGs were identified and these were significantly involved in canonical pathways that contribute to the difference of pathogenesis and the biology between TNBC and low ER-positive breast cancers. Higher expression of *MED12* and *ESR2* could be helpful in examining potential role of tamoxifen therapy in TNBC.

305 Missing Targets After Nipple-Sparing Mastectomy: A Multidisciplinary Approach to Avoid an Undesirable Outcome

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Background: Mastectomy is generally performed without the guidance of wire or radioactive seed localization based on the premise that most of the accessible breast parenchyma and the lesion are removed. Studies have shown that benign breast tissue can be retained to some extent in the patient in certain anatomic locations particularly after nipple-sparing mastectomy (NSM). While the long-term consequence of this phenomenon is unknown, the immediate concern is to ensure complete removal of the tumor. We encountered NSM cases in which the tumor or the tumor site localized by the marker clip was not identified in the specimen.

Design: We prospectively monitored all the mastectomy specimens at our institution in the past 30 months for missing marker clips or primary tumors.

Results: We identified four NSM cases out of 569 therapeutic mastectomies (0.7%) performed at our institution, in which the primary tumor or tumor site had been retained in the body. Subsequent corrective surgeries were performed to remove the tumor or marker clips. The retained lesions in our series were located peripheral to the breast gland: Deep in the inner quadrants in two of the patients (cases 1 and 4); axillary tail of the upper outer quadrant in one patient (case 3) and the superficial subcutaneous tissue of the lower outer quadrant in one patient (case 2). The demographic and clinicopathologic characteristics of the four patients are listed in Table 1.

Age (years)	Location/ Laterality	Tumor to Nipple (cm)	Depth	Diagnosis/ Stage	Image Guidance	Surgery	Interval to Corrective Surgery
37	9:00/L	7	Deep	IDC/ pT1c	Ultrasound	NSM with implant reconstruction	3 months
56	5:00/L	5	Superficial	DCIS/ pTis	MRI	NSM with DIEP flap reconstruction	4 months
42	1:00/L	7	Axilla	IDC/ pT1b	Ulstrasound	NSM with implant reconstruction	2 weeks
48	4:00/R	8	Deep	IDC/ pT2	Unknown	NSM with implant reconstruction	2 weeks

(IDC: Invasive ductal carcinoma; DCIS: Ductal carcinoma in situ; MRI: Magnetic resonance imaging; NSM: Nipple sparing mastectomy; DIEP: Deep inferior epigastric perforators).

Conclusions: In NSM cases where the lesions are located peripheral to the breast gland, we suggest consideration of preoperative radiographic guidance and postoperative specimen imaging for marker clips to avoid the risk of retained cancer and undesirable corrective surgery.

306 Whole Transcriptome Analysis Identifies Upregulated Genes and Pathways Differentially Expressed in Ductal Carcinoma In Situ Mimicking Usual Ductal Hyperplasia

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Background: Ductal carcinoma in situ (DCIS) can display morphologic features overlapping those of usual ductal hyperplasia (UDH). Misdiagnosis of the former, which we designate herein as "DCIS with UDH-like morphology" (DUM), is harmful as the managements of DCIS and UDH are radicaly different. To dissect the molecular drivers of DUM and identify new biomarkers, we performed whole transcriptome analysis of DUM and compared them with patient-matched UDH in the same specimen.

Design: We prospectively identified two cases of DUM with their corresponding UDH. We performed laser capture microdissection (LCM) from five 10-µm-thick sections to obtain pure samples of each respective lesion from formalin-fixed, paraffin-embedded (FFPE) tissue. We performed whole transcriptome analysis of each sample. RNASeq libraries were prepared using the Clontech SMARTer Stranded Total RNA-Seq Kit library prep, following the manufacturer's protocol. The libraries were loaded on high output HiSeq 2500 flow cells generating 60-80 million reads per sample. Data were analyzed using our in-house bioinformatics pipeline including Database for Annotation, Visualization and Integrated Discovery (DAVID) and Gene set enrichment analysis (GSEA).

Results: The DUM cases were significantly enriched for genes previously identified as overexpressed in APC pathway, genes upregulated in breast cancer metastasis to the brain and genes of the transforming growth factor beta-1 (TGFB-1)-Wnt signaling. Some of the top 50 upregulated genes in DUM compared to UDH included (in order of frequency): Insulin-like growth factor binding protein 4 (IGFBP4), keratin 19 (KRT19), cyclin D-1 (CCND1), MAGED2 (MAGE famiy member D2) and FOXA1 (forkhead Box A-1). Some of the top 50 downregulated genes in DUM compared to UDH included (in order of frequency):SFRP1 (secreted frizzeld-related protein 1), PARP4 (poly ADP-ribose polymerase family member 4), MAML2 (mastermind like transcriptional coactivator 2) and NFIB (nuclear factor 1 B).

Conclusions: DUM is a distinct molecular entity with upregulation of genes associated with aggressive behavior in breast cancer. We identified potential novel diagnostic markers for distinguishing UDH from its pre-invasive mimicker. Whole transcriptome analysis of morphologically distinct lesions from the same FFPE sample isolated by LCM provides a powerful tool to identify novel biomarkers in breast cancer.

307 Biomarker Profile Before and After Neoadjuvant Chemotherapy

Jennifer Zeng, Andrea Hernandez, Farbod Darvishian. New York University Medical Center, NY, NY.

Background: Neoadjuvant chemotherapy (NAC) provides the unique opportunity to monitor tumor response. While the response can be evaluated clinically, radiologically or pathologically, the latter is considered the gold standard. The coveted complete pathologic response(pCR) is a powerful prognostic marker and is associated with the most favorable outcome. In the event of incomplete response, the tumor biomarkers, i.e. estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (Her2) and Ki-67, offer desirable targets to assess the impact of NAC on the tumor and assist in planning further therapy. In this study, we explored the effect of NAC on biomarker expression and correlated it with the tumor's molecular subtype.

Design: Between January 2014 and September 2016, we retrospectively searched the archives for patients who were treated with NAC and compared the biomarker profile performed on the core biopsy and the definitive surgical specimen. We defined pCR as no residual invasive carcinoma and no nodal metastasis. The biomarker profiles were classified into 4 categories:Hormone receptor positive (HR+) defined as ER+, PR+, Her2-; Her2+ defined as ER-, PR-, Her2+; HR+/Her2+ defined as ER+, PR+/-, Her2+; and triple negative (TN) defined as ER-, PR-, Her2-.

Results: 58 patients who underwent NAC with biomarkers available on both pre- and post-NAC specimens were identified. Their respective tumors were classified as HR+ (n=23,40%), HR+/her2+ (n=11,19%), Her2+ (n=6,10%) and TN (n=18,31%). Of the 58 patients, 15 achieved pCR (26%). With respect to the molecular classification, of the 15 patients with pCR, 6 (40%) had luminal B tumors (HR+/Her2+, n=5 and HR+ with Ki-67 of 80%, n=1), 4 (27%) had Her2+ tumors and 5 (33%) had TN tumors. No luminal A tumor achieved pCR. Of the 43 patients with residual disease, the pre- and post-NAC biomarker profiles showed an average decrease of 3% (range:-69% to 15%), 10% (range:-98% to 75%) and 18% (range:-85% to 40%) for ER, PR and Ki-67, respectively. Her2 status changed in 4 cases (9%) with in-situ hybridization confirmation as follows:3 cases from Her2- to Her2+ and 1 case from Her2+ to Her2-. Therefore, a categorical shift after NAC was seen in the 4 cases with Her2 status change as follows:3 HR+ tumors changed to HR+/Her2+ and 1 HR+/Her2+ tumor changed to HR+.

Conclusions: NAC induced changes in all the components of the biomarker profile with the greatest change observed in Ki-67 followed by PR and ER. The rate of change in the Her2 status warrants repeating the biomarkers on the post-NAC specimen. Only patients with luminal B, Her2+ and TN tumors achieved pCR.

308 Androgen Receptor Expression Is Higher in CK5/6 Negative versus CK5/6 Positive Triple-Negative Breast Cancers

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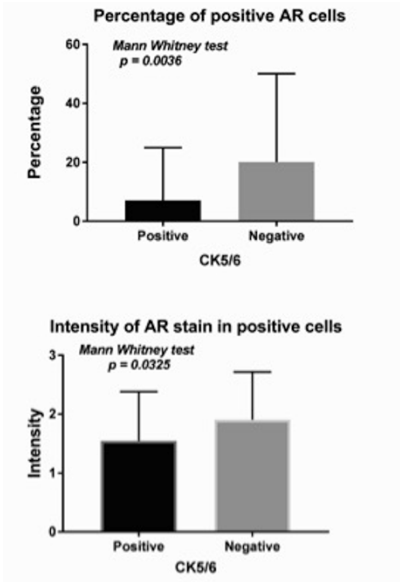
Background: Androgen receptor (AR) expression is observed in about 25% to 75% of triple-negative breast cancers (TNBCs). However the degree of AR expression in TNBC varies widely depending on the cancer subtypes, cutoff for positivity and assay methodology.

Design: TMAs with 291 TNBCs of which 228 were available for immunohistochemical study for AR and CK5/6 expression. The percentage and intensity of both markers were evaluated by two pathologists independently.

Results: The Pearson correlation between two pathologists for AR score was R^2=0.94, CK5/6 positive (+) cases (R^2=0.89), and CK5/6 negative (-) cases (R^2=0.9595). AR expression in TNBCs using 1% and 10% cutoff was 41% and 28% respectively of all TNBCs. CK5/6 expression was seen in 122 of 228 (54%), 106 of 228 (46%) TNBCs. Within AR positive TNBCs using 1% cutoff, in CK5/6+ and CK5/6- groups (56 AR+/CK5/6- and 34 AR+/CK5/6+) there was no statistically significant difference (p = 0.207). Within AR positive TNBCs using 10% cutoff, in CK5/6+ and CK5/6- groups (43 AR+/CK5/6- and 20 AR+/CK5/6+) there was statistically significant difference (p= 0.0247). The percentage and intensity of AR expression in CK5/6 negative cases was higher compared with CK5/6 positive cases (p= 0.0036, p= 0.0325) respectively.



The mean follow-up time in AR+/CK5/6+ was 92 months (mths) (range 24 -180 mths, SD 47.5) versus (vs) a mean follow up time in AR+/CK5/6- of 121 mths (range 48-252 mths, SD 55.2). There was statistically significant difference in survival time for patients with AR+/CK5/6+ TNBCs when compared with AR+/CK5/6- TNBCs (chi square test p value=0.0007).

Conclusions: AR expression is significantly higher in CK5/6- vs CK5/6+ TNBCs. Among AR positive cases, CK5/6- subtype has less favorable outcome compared with CK5/6+.

Cardiovascular Pathology

309 The Role of IgG4 in Acquired Aortic Valve Stenosis: A Study of 110 Consecutive Surgical Resected Aortic Valves

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Background: Mechanisms leading to calcification and fibrosis of aortic valves (AV) are not well understood. Inflammatory and immune processes may contribute to progression of the disease. Anecdotal case reports suggest that immunoglobulin G4 (IgG4)+ plasma cells (PC) may play a role in a subset of degenerative AV disease. No previous study has demonstrated elevated IgG4+ PC in the various types of aortic stenosis.

Design: 110 surgically excised, stenotic AV were consecutively procured. Cases were photographed, measured, decalcified (24-48 hrs), and sampled for histopathologic evaluation. 9 autopsy-derived controls were obtained from individuals without heart disease, hypertension, or an underlying inflammatory condition. Salient clinical and demographic information was abstracted from the medical record. Valves were classified as degenerative, congenitally bicuspid (BAV), or postinflammatory. Immunoglobulin G (IgG) and IgG4 IHC was performed. A single "hot spot" for IgG+ and IgG4+ cells was manually enumerated, and a ratio of IgG4+ to IgG+ cells was calculated.

Results: The cohort (63 men, 47 women) had a mean age of 74 years (range, 47-94). 78 (71%) were classified as degenerative fibrocalcific disease, 22 (20%) as congenitally BAV, and 10 (9%) as postinflammatory.

	Degenerative Fibrocalcific		Congentially Bicuspid		Postinflammatory		Controls (n=9)
	Low IgG4 (n=76)	High IgG4 (n=2)	Low IgG4 (n=19)	High IgG4 (n=3)	Low IgG4 (n=9)	High IgG4 (n=1)	
Age yrs, Mean (range)	77 (53-94)	78.5 (77-80)	67 (47-86)	69 (63-72)	69 (50-80)	74	61 (27-74)
Sex, M (F)	43 (33)	1 (1)	12 (7)	2 (1)	4 (5)	1 (0)	2 (7)
IgG-positive cells, Median (range)	7 (1-150)	38 (24-52)	6 (2-38)	75 (33-141)	13 (1-124)	25	3 (0-11)
IgG4-positive cells, Median (range)	2 (0-15)	16.5 (12-21)	2 (0-8)	61 (17-109)	2 (0-12)	23	0 (0-2)
IgG4:IgG ratio, Median	0.2	0.45	0.21	0.77	0.08	0.92	0

Aortic stenosis was severe in 105 cases and moderate in 5. 27 cases had increased IgG+ cells per high power field (>15), of which 6 showed an IgG4+:IgG+ PC ratio of >0.50 or IgG4+ PC count >20. Of the 6, all had severe stenosis; 3 valves were congenitally BAV, 2 were degenerative, and 1 was postinflammatory. None had clinical features of IgG4-related disease.

Conclusions: This study analyzed stenotic AV for increased IgG4+ PC. A subset of AV (both tricuspid and bicuspid) had increased IgG4+ PC. This finding differs from current literature on the topic and adds to the body of literature published on IgG4-related disease in the AV.

310 The Pathology of Subaortic Membranes: An Analysis of 83 Surgically Resected Cases with Molecular Genetic Correlation

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Background: Subaortic membranes (SAM) are subvalvular collections of fibro(muscular) tissue that result in fixed obstruction. The pathogenesis of subaortic membranes (SAM) remains obscure and while both congenital and acquired forms have been identified, the latter is thought to be more common. Phenotypic overlap exists with other forms of outflow obstruction, such as hypertrophic cardiomyopathy (HCM) and differentiating between them is important given the heritable implications of cardiomyopathic states. Herein, histopathologic and molecular genetic features of a population of 83 surgically resected cases of SAM are evaluated.

Design: Formalin-fixed paraffin embedded (FFPE) tissue was obtained on 83 patients having undergone surgical resection of a discrete or tunnel SAM (2009-2015). Clinical information and hemodynamic data was abstracted from the medical record. Light microscopic features, including myocyte disarray, myocyte hypertrophy, and interstitial fibrosis were semiquantitatively scored. Extracted genomic DNA underwent Agilent SureSelect targeted capture of 54 genes associated with cardiomyopathies, followed by sequencing on the Illumina MiSeq platform. Variants were classified according to established guidelines.

Results: 83 patients (54 women) were included in the study, with a mean age of 49.8 years (range, 4-80). 77 cases of SAM were discrete membranous, while 6 were tunnel-type. Myocyte hypertrophy was absent or mild in 6 cases, moderate in 53 and severe in 23 cases. Interstitial fibrosis was absent or mild in 56 cases, moderate in 25, and severe in 1. Myocyte disarray was absent or mild in 75 cases and moderate in 7. The majority of genetic variants identified were benign polymorphisms; however, 4 pathogenic/likely pathogenic and 77 variants of unknown significance were identified (average, 1.7/case). Of the pathogenic/likely pathogenic variants, mutations in *PTPN11* were present in 2 cases, *MYH7* mutation in 1, and *SOS1* in 1 case. 25 cases were believed clinically to have concomitant HCM, though none of those carried a molecular genetic mutation compatible with such. No histopathologic or clinical parameter appeared to correlate with identified pathogenic mutations.

Conclusions: Hitherto, this is the first systematic survey of SAMs to evaluate their histopathologic features. Additionally, it is the largest series of cases of SAMs to undergo molecular genetic interrogation to evaluate for concomitant cardiomyopathy or syndrome. Histologic findings alone did not appear to be a predictor of underlying genetic variation to help in evaluation for a cardiomyopathic or syndromic state.

311 Arrhythmogenic Cardiomyopathy: A Genotype-Phenotype Correlation of 15 Cases

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Background: Arrhythmogenic cardiomyopathy (AC) is a rare and potential fatal form of heritable cardiovascular disease. Despite important advances in understanding the genetic basis of AC, identification of novel potentially pathogenic mutations remains paramount in the recognition, diagnosis and treatment of at-risk patients. Herein, genetic analysis of 15 AC specimens was performed and correlated with gross and histopathologic features.

Design: Formalin-fixed paraffin embedded tissue was obtained on 15 archived AC cases (2009-2015), meeting established clinical diagnostic criteria for said diagnosis. Clinical information was abstracted from the medical record. Gross and histopathologic features of AC cases were recorded. Extracted genomic DNA underwent Agilent SureSelect targeted capture of 54 genes associated with cardiomyopathies, followed by sequencing on the Illumina MiSeq. Variants were classified according to established American College of Medial Genetics (ACMG) guidelines.

Results: 15 patients (8 men) with a mean age of 48 years (range, 11-69) were included in the study (12 explant; 3 autopsy). 9 patients had right-ventricular (RV) involvement,