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# ABSTRACTS

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## 100 Clinicopathologic and Biological Significance of Altered Phosphatidylinositol 3- Kinase (PI3KCA) Function in Breast Cancer of Nigerian Women

Kikelomo Adeleke[1], Victoria Iyawe[2], Henry Ebili[2], Ayodeji Agboola[3]
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**Disclosures:** Kikelomo Adeleke: None; Victoria Iyawe: None; Henry Ebili: None; Ayodeji Agboola: None

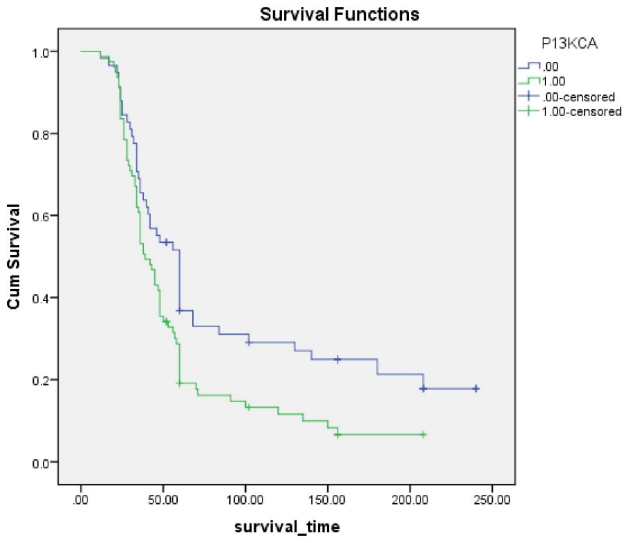
**Background:** PI3KCA plays major regulatory roles in cellular growth, proliferation, transformation, adhesion, motility, survival and apoptosis. About 27% of human breast tumours carry gain-of-function mutations in the *PIK3CA* gene and these genetic abnormalities result in increased lipid kinase activity of the catalytic subunit of the enzyme. A proper understanding of this pathway is therefore important in developing therapeutic modalities for the management of breast cancer. Previous studies have shown that the breast cancer in women of African descent is highly aggressive and associated with poor prognostic indices. However there is paucity of information on the prognostic significance of PI3KCA protein expression among women with breast cancer in Nigeria. This study therefore aims to determine PI3KCA expression in breast cancers of women in Nigeria and its prognostic significance.

**Design:** Immunohistochemistry was carried out on tissue microarrays constructed from 271 formalin-fixed paraffin embedded tissue blocks from women with breast cancer. Data relating to survival were categorized as breast cancer specific survival (BCSS), defined as the interval (in weeks) from the date of the primary treatment to the time of death, and disease free interval (DFI), defined as the interval (in weeks) from the date of the primary treatment to the first loco-regional recurrence or distant metastasis. The patients were followed up for at least 60 months (260 weeks). Chi square analyses were used to test relationship between PI3KCA, clinicopathological parameters and other biomarkers. The Kaplan–Meier survival method and the log-rank test were used for survival curves. Test of statistical significance was set at $p < 0.05$.

**Results:** 64.3% (198) of the patients were 50 years and below. Positive expression of PI3KCA was present in 50.6% (137) of breast tumors and this was associated with p53 over-expression, high tumor grade, triple negative status of the tumors and lack of expression of hormone receptors (estrogen and progesterone receptors).(table 1). Survival analysis shows that patients with positive expression of PI3KCA had poorer BCSS compared to those that had negative expression(p=0.008). (figure 1)

| Variables | PI3KCA expression Negative | Positive | $x^2$ | p-value |
|---|---|---|---|---|
| **Tumour grade** | | | | |
| I | 3(2.2%) | 3(2.2%) | 13.66 | 0.001 |
| II | 98(73.1%) | 71(51.8%) | | |
| III | 33(24.6%) | 63(46.0%) | | |
| **Triple negative tumour** | | | | |
| No | 57(58.8%) | 43(39.4%) | 7.66 | 0.006 |
| Yes | 40(41.2%) | 66(60.6%) | | |
| **Estrogen receptor** | | | | |
| Negative | 81(67.5%) | 103(81.1%) | 6.01 | 0.014 |
| Positive | 32.5%) | 24(18.9%) | | |
| **Progesterone receptor** | | | | |
| Negative | 69(68.3%) | 93(84.5%) | 7.78 | 0.005 |
| Positive | 32(31.7%) | 17(15.5%) | | |
| **p53** | | | | |
| Negative | 42 (60.9%) | 48 (35.8%) | 11.58 | 0.001 |
| Positive | 27(39.1%) | 86 (64.2%) | | |

Figure 1 - 100




**Conclusions:** This study shows that PI3KCA protein expression is high in Nigeria breast cancers and it is also an indicator of aggressive tumour phenotype. Nigerian women with breast cancer are likely to benefit from therapeutic modalities involving PI3KCA inhibitor.

## 101 Monosomy of Chromosome 17 in Breast Cancer: Experience at a Single Academic Institution

Rana Ajabnoor[1], Bradley Turner[1], Marcus D'Aguiar[1], David Hicks[1], Huina Zhang[1]
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**Disclosures:** Rana Ajabnoor: None; Bradley Turner: None; Marcus D'Aguiar: None; David Hicks: None; Huina Zhang: None

**Background:** Chromosome17 (Ch17) plays a significant role in breast carcinogenesis, as it expresses the HER2 oncogene. Fluorescent in situ hybridization (FISH) assesses Ch17 status by the number of HER2 copies per Ch17 in relation to the number of centromere copies (CEP17) per Ch17. The 2018 CAP/ASCO recent recommendations specify breast cancer patients with monosomy 17 as HER2 FISH group 2 (HER2 copy number/CEP17 ratio > or equal 2.0 and average HER2 signal/cell < 4.0). Previously these patients were called HER2 positive; however, the recent recommendations suggest they now be called HER2 negative if the immunohistochemistry (IHC) is equivocal or negative. The incidence of this group is rare (0.7%). Whether or not group 2 patients will benefit from HER2 targeted therapy with Herceptin or not is still controversial, and the literature on this subject is limited. Our study analyzes the outcome of HER2 targeted therapy on group 2 patients, in correlation with other clinicopathologic features.

**Design:** Thirteen patients with group 2 HER 2 FISH results with equivocal or negative IHC and available follow-up between 2013 and 2018 were identified from the pathology database at our institution. Clinicopathological features including age, Ki67, histological grade, HER2 therapy status, and disease outcome were analyzed (Table 1).

**Results:** There was a trend towards a significantly increased Ki-67 (p=0.01) and a higher grade in patients that metastasized in our group 2 population (Table 1). However, there did not seem to be any trend towards metastasis associated with receiving HER-2 therapy (Table 1).

**Table1:** Correlation of clinocopathologic features and outcome

| Variables | Group2 | Follow up of Disease | |
|---|---|---|---|
| | | NED* | MET** |
| N | 13 | 9 (69%) | 4 (31%) |
| Age (mean) | 58 | 54 | 55 |
| Ki67 (mean) | 45% | 37%*** | 78%**** |
| Histologic Grade | | | |
| G1-G2 (N) | 9 (69%) | 7 (78%) | 2 (22%) |
| G3 (N) | 4 (31%) | 2 (50%) | 2 (50%) |
| Herceptin | | | |
| Treated (N) | 10 (53%) | 7 (70%) | 3 (30%) |
| Not treated (N) | 3 (16%) | 2 (67%) | 1 (33.33%) |

Table 1: * NED: no evidence of disease; **MET metastatic disease; ***N= 7; **** N = 2

**Conclusions:** In conclusion, in our population, Herceptin targeted therapy does not appear to have an impact on disease outcome in monosomy 17 breast cancer patients, supporting the recent CAP/ASCO recommendations that group 2 patients now be called HER2 negative. However, our data is limited and additional study on the effect of Herceptin targeted therapy in a larger patient population is needed to determine the value of Herceptin targeted therapy in group 2 patients.

## 102 Breast Cancers with 'Co-amplified/Polysomy' of HER2 FISH Result: Clinicopathological Features and Follow-Up Outcomes

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*[1]University of Rochester Medical Center, Rochester, NY*

**Disclosures:** Rana Ajabnoor: None; Bradley Turner: None; Marcus D'Aguiar: None; David Hicks: None; Huina Zhang: None

**Background:** Chromosome17 (Ch17) plays a significant role in breast carcinogenesis, as it expresses the HER2 oncogene. Fluorescent in situ hybridization (FISH) assesses Ch17 status by the number of HER2 copies per Ch17 in relation to the number of centromere copies (CEP17) per Ch17. The 2018 CAP/ASCO recent recommendations specify breast cancer patients with 'Co-amplified/Polysomy' of HER2 as HER2 FISH group 3 (HER2 copy number/CEP17 ratio < 2.0 and average HER2 signal/cell > or equal 6.0). These patients are still HER2 positive according to the recent CAP/ASCO recommendations; however, previous studies have reported worse disease free survival in group 3 patients treated with HER2 targeted therapy with Herceptin. The incidence of this group is rare (0.5%). Whether or not group 3 patients will benefit from HER2 targeted therapy or not is still controversial, and the literature on this subject is limited. Our study analyzes the outcome of HER2 targeted therapy on group 3 patients, in correlation with other clinicopathologic features.

**Design:** Seventeen patients with group 3 HER 2 FISH results and available follow-up between 2013 and 2018 were identified from the pathology database at our institution. Clinicopathological features including age, stage, HER2 therapy status, and disease outcome were analyzed (Table 1).

**Results:** Patients who were treated with Herceptin targeted therapy had worse disease outcome compared to patients who were not treated with Herceptin targeted therapy (Table 1). 90% (4/5) of the patients that had metastatic disease were stage III-IV, and all stage III-IV patients with metastatic disease (100%) received Herceptin targeted therapy. 75% (9/12) of the patients that did not have metastatic disease were stage I-II. Five of these stage I-II patients that did not have metastatic disease (56%), received Herceptin targeted therapy.

**Table1:** Correlation of clinocopathologic features and outcome

| Variables | Group 3 | Follow up of Disease | |
|---|---|---|---|
| | | NED* | MET** |
| **N** | 17 | 12 (71%) | 5 (29%) |
| **Age (mean)** | 65 | 63 | 66 |
| **# of HER2 copies (mean)** | 7 | 7 | 8 |
| **Stage** | | | |
| **I-II*** ** | 10(57%) | 9 (90%) | 1 (10%) |
| **III-IV** | 6 (35%) | 2 (33%) | 4 (67%) |
| **Herceptin** | | | |
| **Treated** | 13 (76%) | 8 (62%) | 5 (38%) |
| **Not treated** | 4 (23%) | 4 (100%) | 0 (0%) |

* NED: No evidence of disease; ** MET: metastatic disease; ***N = 1 case with no stage

**Conclusions:** In conclusion, in our population, Herceptin targeted therapy may not be of benefit for patients with more advanced stage (III-IV) breast carcinoma, but may be beneficial in patients with lower stage (I-II) breast carcinoma; however, our data is limited and additional study on the effect of Herceptin targeted therapy in a larger patient population is needed to determine the value of Herceptin targeted therapy in group 3 patients.

## 103 HER2 ISH Group 4 Cases: Additional testing and likelihood of identifying a positive result

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**Disclosures:** Hiba Al Dallal: None; Mary Ann Sanders: None

**Background:** The 2018 ASCO/CAP Guidelines eliminated the result of equivocal for cases with an average HER2 copy number of ?4 and <6 signals per cell with a HER2/CEP17 ratio of <2, renamed these as HER2 ISH Group 4, and provided an algorithm of additional testing

resulting in either a positive result or a negative result with a comment. The algorithm requires HER2 immunohistochemistry (IHC) results and in cases where HER2 IHC is equivocal, advises recounting ISH in areas with IHC 2+ staining. The guidelines also state that a new HER2 test may be ordered on the excision specimen if the core biopsy result is equivocal for HER2 testing by both ISH and IHC. The purpose of this study is to evaluate the effectiveness of immunohistochemistry or testing a different specimen in resolving HER2 ISH Group 4 cases.

**Design:** Our data base was searched for consecutive cases of breast cancer where HER2 FISH was performed over a two year period. A second search was performed selecting only cases that were ISH Group 4. Corresponding pathology reports were reviewed and clinicopathologic data recorded. FISH was performed using the PathVysion Kit (Her2neu/ CEP17 probes) according to the manufacturer's instruction. In cases where HER2 immunohistochemistry was not previously performed, the block, if available, was retrieved and HER2 IHC was performed using the DAKO Hercep Test Kit according to the manufacturer's instruction.

**Results:** A total of 1687 HER2 FISH cases were identified and of these 69 cases (4%) were ISH Group 4. HER2 IHC was performed on the same block in 59 of 69 cases (86%) and of these 21 (36%) were negative (0 or 1+), 38 (64%) were equivocal (2+) and zero cases were positive (3+). Eighteen cases had a new specimen tested for HER2 and of these 6 (33%) were negative, 3 (17%) were positive, 7 (39%) were ISH Group 4, and 2 (11%) were ISH Group 3 (HER2 copy ? 6 and HER2/CEP17 <2).

**Conclusions:** The majority of ISH Group 4 cases were HER2 IHC equivocal, and when a new specimen was tested half of cases were either ISH Group 4 or ISH Group 3 requiring additional testing. Moreover, rare cases were HER2 positive with additional testing. Therefore, our data suggests that ISH Group 4 cases will result in a significant increase in testing with a low probability of identifying HER2 positive cases and an increased likelihood that most cases will be resolved to a negative result with a comment.

## 104 Invasive Breast Carcinoma with Apocrine Differentiation: HER2+ Phenotype Supersedes Triple-Negative Status to Stratify Tumor Grade and Prognostic Factors

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**Disclosures:** Ellen Alexander: None; Husain Sattar: None; Jeffrey Mueller: None; Thomas Krausz: None; Anna Biernacka: None

**Background:** *Carcinoma with apocrine differentiation* comprises a spectrum of in situ and invasive breast cancers that recapitulate native mammary apocrine epithelium and often exhibit an estrogen receptor (ER)-negative/androgen receptor (AR)-positive profile with HER2 amplification in a subset of tumors. Peer reviewed data supports both improved and worsened clinical behavior within these tumors. When to report - and how to reproducibly recognize - apocrine features lack consensus criteria, which impede accurate epidemiologic assessment and limit the ability to understand the biologic underpinnings and clinico-pathologic significance of these lesions.

**Design:** We performed a retrospective assessment of 41 cases of invasive breast carcinoma classified as "apocrine". Five pathologists reviewed the H&E slides to confirm the diagnosis (kappa 0.902). ER/HER2 status and pathologic parameters were obtained from diagnostic reports. Tumors were subtyped as ER+/HER- (n=10), ER+/HER+ (n=1), ER-/HER+ (n=9), or ER-/HER2- (TNBC) (n=21) and further stratified by SBR grade. Immunohistochemistry for AR (DAKO, AR441) was performed; 39/41 cases (95%) were positive with a threshold of ≥10% nuclear staining.

**Results:** The majority of cases exhibited high SBR grade (39/41 SBR grades 2 and 3). HER2 amplification was associated with higher SBR grade compared to TNBC (HER2+ SBR grade III vs TNBC SBR grade II, P=0.0002). HER2 amplification was additionally associated with increased tumor size (HER2+ 2.0cm vs TNBC 1.1cm, p=0.047), tumor necrosis (HER2+ 90.1% vs TNBC 48%, p<0.0001), and lymph node metastases (HER2+ 90.1% vs TNBC 43%, p=<.0001) compared to TNBC. While higher tumor grade was associated with tumor size, tumor necrosis, and lymph node metastases on univariate analysis, this effect was nullified when controlling for HER2 status.

**Table 1**: Tumor grade and subtypes with associated pathologic parameters for invasive apocrine carcinoma

| | | | SBR Grade | | | | Pathologic Features by Receptor Profile | | |
|---|---|---|---|---|---|---|---|---|---|
| | | All cases (n=41) | I (n=2) | II (n=18) | III (n=21) | | Tumor size (cm) | Tumor necrosis | Lymph node Metastases |
| | ER+/HER- | 24.3% | 50% | 30% | 19.0% | | 1.3 | 40% | 40% |
| | ER+/HER+ | 2.4% | 0% | 0% | 4.8% | | 2.4 | 100% | 100% |
| | ER-/HER+ | 22.0% | 0% | 0% | 42.9% | | 1.9 | 89% | 89% |
| | ER-/HER2- | 51.2% | 50% | 70% | 23.8% | | 1.1 | 48% | 43% |
| *Pathologic Features by SBR Grade* | *Tumor size (cm)* | *1.3* | *1.1* | *1.1* | *1.8* | | | | |
| | *Lymph node metastases* | *39%* | *0%* | *20%* | *67%* | | | | |
| | *Tumor necrosis* | *54%* | *0%* | *25%* | *81%* | | | | |

The percentages listed under grade/profile subtypes reflect percentage of ER/HER subtype within each SBR grade. Tumor size and lymph node status were evaluated in 28 cases. Tumor necrosis was evaluated in all cases. *Pathologic features (tumor size, lymph node status, tumor necrosis) in relation to SBR lose significance after controlling HER2 status.*

**Conclusions:** Invasive apocrine carcinoma demonstrates a predilection towards high SBR grade. All invasive apocrine carcinomas with HER2 amplification were SBR grade III, whereas the majority to triple-negative tumors were SBR grade II. HER2 status was significantly associated with high-risk pathologic features, compared to triple-negative status, independent of tumor grade. These findings may account for the variable pathobiology and clinical behavior observed in breast carcinomas with apocrine differentiation; HER2 overexpressing apocrine carcinomas may behave more aggressively than their triple-negative counterparts.

## 105 Tumor Infiltrating Lymphocytes and PD-L1 Expression Characterize a Divergent Immune Microenvironment in Invasive Apocrine Carcinoma of the Breast

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**Disclosures:** Ellen Alexander: None; Thomas Krausz: None; Anna Biernacka: None

**Background:** Invasive apocrine carcinoma (IAC) is a rare, primary breast cancer characterized by apocrine morphology with a frequent estrogen (ER) negative/androgen (AR) positive receptor profile, and HER2 amplification in a subset of cases. Molecular profiling of these tumors demonstrates considerable overlap with Luminal AR-type TNBC (LAR). Unlike other TNBC subtypes, LAR exhibits a molecular profile akin to Luminal A/B type breast cancer and confers an improved prognosis, despite ER/PR negativity. These findings challenge the interpretation of prognostic features, such as tumor infiltrating lymphocytes (TILs) and PD-L1 expression, which portend a better prognosis in triple-negative and HER2 amplified breast cancer. The immune microenvironment has yet to be described for IAC and may aid the interpretation of the divergent features seen within these tumors.

**Design:** We identified 40 invasive carcinomas with apocrine morphology (39 ductal, 1 lobular). Tumors were stratified by ER/HER2 status and SBR grade. Peritumoral stromal TILs were quantified per International Immunooncology Biomarkers Working Group guidelines: 0 – 10% (low, A), 11 – 40% (mild-moderate, B), >41% (diffuse, C). Immunohistochemistry for PD-L1 (EIL3N antibody) was performed for each case; an H-score was calculated for tumor cell staining, with a threshold of positivity set for values above the mean. Strong PD-L1 staining in TILs was recorded as positive.

**Results:** Variable degrees of TILs were seen within the study sample: 40% (16/40) low TILs (group A), 32.5% mild-moderate TILs (group B), and 27.5% diffuse TILs (group C) within the peritumoral stroma; the normal distribution curve showed positive-skew, indicating a predilection towards lower TILs, particularly within lower grade tumors. Tumor cell PD-L1 expression was seen in 15% of cases (6/40); PD-L1+ carcinomas had a higher degree of TILs (mean PD-L1+ TILs 63% vs PD-L1- TILs 29%, p=0.022517) and a higher SBR grade (PD-L1+ grade III vs PD-L1- grade II, p=0.040641). PD-L1+ TILs were seen in 52.5% of cases and correlated with stromal TILs (diffuse TILs 90.9% vs low TILs 25%, p=0.000758).

| | Peritumoral TILs | | PDL1 | | Receptor Status | | | SBR Grade |
|---|---|---|---|---|---|---|---|---|
| | Stromal TILs | Group | TILs | Tumor* | ER+ | HER+ | TNBC+ | |
| 1 | 10% | A | | 0 | | | X | 2 |
| 2 | 60% | C | X | 9 | | | X | 2 |
| 3 | 5% | A | | 0 | X | | | 3 |
| 4 | 90% | C | X | 120 | | X | | 3 |
| 5 | 20% | B | X | 24 | | X | | 3 |
| 6 | 15% | B | | 0 | | | X | 2 |
| 7 | 20% | B | | 0 | X | X | | 3 |
| 8 | 50% | C | X | 20 | | X | | 3 |
| 9 | 80% | C | X | 95 | | X | | 3 |
| 10 | 60% | C | X | 41 | | | X | 2 |
| 11 | 15% | B | | 0 | | | X | 2 |
| 12 | 80% | C | X | 130 | | | X | 3 |
| 13 | 15% | B | X | 10 | X | | | 2 |
| 14 | 10% | A | X | 0 | | | X | 2 |
| 15 | 10% | A | | 0 | | | X | 2 |
| 16 | 5% | A | | 0 | | | X | 1 |
| 17 | 50% | C | X | 7 | X | | | 2 |
| 18 | 20% | B | | 0 | | | X | 2 |
| 19 | 20% | B | X | 0 | X | | | 2 |
| 20 | 10% | A | | 0 | | | X | 2 |
| 21 | 20% | B | X | 3 | | | X | 3 |
| 22 | 40% | B | X | 0 | X | | | 2 |
| 23 | 10% | A | | 0 | X | | | 2 |
| 24 | 5% | A | X | 0 | | | X | 2 |
| 25 | 10% | A | | 5 | | | X | 2 |
| 26 | 90% | C | X | 5 | | X | | 3 |
| 27 | 10% | A | | 0 | | | X | 2 |
| 28 | 20% | B | | 0 | | | X | 3 |
| 29 | 80% | C | | 0 | | | X | 2 |
| 30 | 5% | A | X | 0 | | | X | 1 |
| 31 | 15% | B | X | 0 | X | | | 2 |
| 32 | 50% | C | X | 4 | X | | | 3 |
| 33 | 30% | B | X | 0 | X | | | 2 |
| 34 | 5% | A | | 0 | | | X | 3 |
| 35 | 80% | C | X | 0 | | X | | 3 |
| 36 | 5% | A | | 8 | | X | | 3 |
| 37 | 10% | A | | 0 | | X | | 3 |
| 38 | 25% | B | | 0 | | | X | 3 |
| 39 | 5% | A | | 0 | | X | | 3 |
| 40 | 5% | A | X | 0 | X | | | 3 |

**Table 1**: Tumor-infiltrating lymphocytes, PD-L1 expression, receptor profile, and tumor grade of invasive apocrine carcinomas

*Tumor cell PD-L1 staining is reported as an H-score

**Conclusions:** Invasive apocrine carcinoma exhibits a spectrum of immunogenicity, a subset of which mirrors the observed frequency of TILs and PD-L1 expression seen in HER2-amplified and triple-negative breast cancer. PD-L1 expression in tumor cells and TILs is associated with SBR grade. Further investigation into the prognostic implications and molecular correlates to these findings is warranted.

## 106 Glucocorticoid Receptor (GR) Expression in Invasive Apocrine Carcinoma of the Breast: GR Expression Correlates with Extent of Apocrine Differentiation, ER Expression, and HER2 Status

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**Disclosures:** Ellen Alexander: None;Thomas Krausz: None; Anna Biernacka: None

**Background:** Steroid receptors for estrogen (ER) and androgen (AR) play essential roles in breast and prostate cancer, respectively, the function of which is modified by glucocorticoid receptor (GR). In ER+ breast cancer and AR+ prostate carcinoma, GR signaling inhibits tumor cell growth, where as GR promotes tumor cell survival and invasion when these receptors are lost. Pure apocrine carcinoma (PAC) is a rare, ER-negative, AR-positive breast cancer with extensive apocrine morphology, thought to be driven by AR signaling. Different degrees of apocrine differentiation and AR positivity are also observed in ER+ breast cancers, and in cancers with HER2 overexpression. GR signaling has not been defined in the specific context of these lesions and may offer insight into the biological underpinnings of these tumors.

**Design:** We conducted a retrospective review of 41 cases of invasive apocrine carcinoma, identified by reported diagnosis and confirmed on pathologic assessment. The extent and pattern of apocrine differentiation was recorded as: none, focal, variable, or diffuse. Tumors

were subtyped as ER+/HER- (n=10), ER+/HER+ (n=1), ER-/HER+ (n=9), and TNBC (n=21) and stratified by SBR grade. Immunohistochemical stains for AR (DAKO, AR441) and GR (Cell Signaling, D8H2) were performed. The positivity threshold for AR was ≥5% strong staining in the areas of apocrine differentiation. GR staining was recorded as: none, focal (localized to area of differentiation), variable (intensity/ distribution), and diffuse (strong throughout).

**Results:** AR was positive in 95% of cases with apocrine morphology. Different degrees of apocrine differentiation were observed: focal (14.6%), variable (34.1%), and diffuse (48.8%). GR immunoreactivity localized to areas with apocrine morphology with concordance between patterns of apocrine differentiation and GR expression as follows: focal-focal (100%; p=0.0156), variable-variable (50%; p=0.5), and diffuse-diffuse (70%, p=0.0318). Diffuse apocrine morphology with concurrent diffuse GR expression was only seen in TNBC. Variable GR was highly associated HER2 expression compared to TNBC (HER2 69.2% vs TNBC 0%, p<0.0001). ER positivity was inversely associated with GR expression (p<0.05).

| | | GR Expression | | | | | Phenotypic Variant | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | None (n=4) | Focal (n=6) | Variable (n=13) | Diffuse (n=20) | | ER+/HER2- | ER+/HER2+ | ER-/HER+ | ER-/HER- |
| **Apocrine Differentiation** | None (n=1) | 1 | 0 | 0 | 0 | | 1 | 0 | 0 | 0 |
| | Focal (n=6) | 0 | 6 | 0 | 0 | | 0 | 6 | 0 | 0 |
| | Variable (n=14) | 3 | 0 | 7 | 4 | | 8 | | 4 | 2 |
| | Diffuse (n=20) | 0 | 0 | 6 | 14 | | 1 | 1 | 4 | 14 |
| | | | | | | | | | | |
| **Phenotypic Variant** | ER+/HER2- | 4 | 0 | 4 | 2 | | | | | |
| | ER+/HER2+ | 0 | 0 | 1 | 0 | | | | | |
| | ER-/HER+ | 0 | 0 | 8 | 1 | | | | | |
| | ER-/HER- | 0 | 6 | 0 | 15 | | | | | |

**Table 1**: GR expression in relation to patterns of aprocrine differentiation and association with receptor subtypes in invasive breast carcinomas

**Conclusions:** GR expression and apocrine morphology colocalize in invasive breast carcinoma. Specific patterns of GR expression and apocrine morphology are strongly associated with ER and HER2 status, and TNBC phenotypes. Further investigation into the prognostic implications is warranted.

## 107 Low Grade Ductal Carcinoma in Situ: How Strongly Do We Agree?

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**Background:** Steadily growing evidence has shown that a proportion of patients with screen-detected ductal carcinoma in situ (DCIS) are overly treated. Subsequently, clinical trials in Europe, United States and Australia have launched with the intent of assessing the safety of treating low grade DCIS with an active surveillance approach rather than the current standard of care, which includes ablative surgery with/out radiation. These trials are dependent on pathologists' interpretation of nuclear features in separating low grade from the pool of DCIS. Herein, we aim to determine the inter-observer reproducibility in diagnosing low grade DCIS.

**Design:** Slides of 300 pure DCIS cases pooled from two institutions were retrieved, the foci of DCIS were marked and the original nuclear grades at the time of sign-out were collected. Holland, Van Nuys and modified Black nuclear grade criteria for low grade DCIS were given to four participating pathologists (three expert breast pathologists and one junior pathologist) prior to histologic interpretation. The pathologists analyzed the same set of marked slides, blinded to the original reported grade of DCIS. The participants were required to identify low grade DCIS from the set of slides provided. Separating intermediate and high grade was not required (categorized as non-low). Kappa values were used to assess agreement between pathologists.

**Results:** The mean age of patients is 58.3 (±12).Of the 300 DCIS cases, 16% (50 cases) were originally diagnosed as low grade. The frequency of low grade amongst participating pathologists ranged from 11.3% to 23%. The inter-observer agreement was considered moderate when all participants were included as well as when the junior pathologist was excluded (kappa= 0.535 and 0.582, respectively). On average, 38% (19/50) of the DCIS cases originally diagnosed as low grade, were upgraded by the participants, while 12.5% (20/159) intermediate-grade DCIS were downgraded. All low grade DCIS cases diagnosed by participating pathologists were ER positive.

**Conclusions:** Pathologists' reproducibility on diagnosing low grade DCIS showed moderate agreement. Experience does not seem to be an influencing factor on improving reproducibility. A more consistent method that includes molecular classification may be the better way to stratify patients for protocol enrollment in low risk DCIS trails of active surveillance, given that morphologic classification is hindered by its inherent subjectivity.

## 108 2018 ASCO/CAP Her2 Testing Guidelines in Breast Cancer. Impact of the Updated Guidelines on Breast Carcinomas Originally Classified as Her2 Equivocal – A Retrospective Analysis

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**Background:** Invasive breast carcinomas with equivocal Her2 status are a challenge to clinicians when making targeted therapy treatment decisions. The American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP) recently issued 2018 Her2 testing guidelines that essentially eliminate the equivocal category. Fluorescence in-situ hybridization (FISH) scoring criteria are now divided into 5 groups that define positive and negative Her2 results. Groups 2, 3 and 4 require concomitant review of immunohistochemistry (IHC) for a final Her2 status designation.

**Design:** We conducted a retrospective review of our institutional Her2 data to include all invasive breast carcinoma cases classified with the previous ASCO/CAP guidelines from 2013. These results from both IHC and dual probe FISH tests were reviewed and tabulated. The Her2 status of the breast carcinomas were then classified into groups 1-5, as outlined in the new 2018 ASCO/CAP guidelines (*Figure 1*).

**Results:** The study included 1493 invasive breast carcinomas from 2013-2017. Based on IHC, 149 cases (10%) were Her2 positive, 969 (64.9%) were negative and 375 (25.1%) were equivocal. Reflex FISH testing performed on the IHC equivocal cases showed the following: 39 (10.4%) were amplified, 305 (81.3%) were non-amplified, and 31 (8.3%) were FISH equivocal. Applying the 2018 guidelines, all 31 of these previously equivocal cases were re-designated as Her2 negative: 29/31 cases were classified as Group 4 (IHC2+) and 2/31 as Group 5. *Table 1* summarizes the results.

Table 1.

**Dual Probe FISH Testing on Equivocal Her2 (IHC 2+)**
**Comparison of Her2 designation according to 2013 and 2018 ASCO/CAP guidelines**

| **2013 Guidelines** | **FISH Amplified** | **FISH Non Amplified** | **FISH Equivocal** | | **Total** |
|---|---|---|---|---|---|
| | **39 (10.4%)** | **305 (81.3%)** | **31 (8.3%)** | | **375 (100%)** |
| **2018 Guidelines** | **Her2 Positive** | **Her2 Negative** | **Her2 Negative** | **Her2 Negative** | |
| | **39 (10.4%)** | **305 (81.3%)** | **29 (93.5%)** | **2 (6.5%)** | |
| | *37 (Group 1); 2 (Group 3)* | *301 (Group 5); 4 (Group 4)* | *(Group 4)* | *(Group 5)* | |

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| Summary of HER2 ISH Diagnostic Criteria for Dual Probe Assay | |
|---|---|
| Her2 Positive | Her2 Negative |
| Group1 | Group 2 AND concurrent IHC 0-1+ or 2+ |
| Group 2 AND concurrent IHC 3+ | Group 3 AND concurrent IHC 0-1+ |
| Group 3 AND concurrent IHC 2+ or 3+ | Group 4 AND concurrent IHC 0-1+ or 2+ |
| Group 4 AND concurrent IHC 3+ | Group 5 |

| Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|
| Ratio ≥2.0<br>≥4.0 signals/cell | Ratio ≥2.0<br><4.0 signals/cell | Ratio <2.0<br>≥6.0 signals/cell | Ratio <2.0<br>≥4.0 and <6.0 signals/cell | Ratio <2.0<br><4.0 signals/cell |

Adapted from "ASCO/CAP Her2 Testing in Breast Cancer Guideline: 2018 Focused Update, Summary of Changes and Definitions." http://www.cap.org/ShowProperty?nodePath=/UCMCon/Contribution Folders/WebContent/pdf/2018-her2-breast-summary-changes.pdf. Accessed 26 September 2018.

**Conclusions:** Our institutional data showed only 2% (31/1475) of invasive breast carcinomas were originally classified as Her2 equivocal under the 2013 guidelines. Although this patient cohort was small, it complicated treatment decisions and often necessitated additional Her2 testing on other patient specimens and/or testing with different FISH probes. By eliminating the equivocal Her2 status, the 2018 CAP/ASCO Her2 guidelines simplified the decision to treat with targeted therapy. Our study demonstrated that all of the breast carcinomas originally classified as equivocal were re-designated as Her2 negative under these new 2018 guidelines. These findings reassure pathologists and clinicians, given that patients who did not receive targeted therapy due to an equivocal Her2 status were not unduly denied treatment by today's latest guidelines.

## 109 Immunophenotypic Features of Invasive Breast Cancers with High Mitotic Index Following Neoadjuvant Chemotherapy

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**Background:** High mitotic count following neoadjuvant chemotherapy (NAC) has been shown to be an independent prognostic factor in breast cancer. We sought to analyze the immunophenotype of these tumors.

**Design:** Of stage I-III breast cancer patients who received neoadjuvant therapy (N=427; 2000-2016), those with available material and sufficient residual invasive carcinoma (ypT1a(m) or greater size, at least 15% tumor cellularity) following NAC (143 tumors from 142 patients; 33.2%) were assessed for mitotic count/10hpf and other clinical-pathologic features. Tissue microarrays were created to assess immunoprofiles of tumors with high mitotic index (score 3) (N=32, 7.5%).

**Results:** Post-NAC tumor size ranged from 0.3-17 cm (median 3.1). 127 (88.8%) were invasive ductal carcinoma (IDC) and 16 (11.2%) invasive lobular carcinoma (ILC). Special features were seen in 9 IDC: mucinous (3), metaplastic (3), micropapillary (2) and neuroendocrine (1). 64 (44.8%) were grade 2, 62 (43.4%) grade 3 and 17 (11.9%) grade 1. Mitosis/10hpf ranged from 0-132 (median 2).

Patients with high mitotic index were younger at diagnosis, more frequently African American, had higher recurrence, metastasis and death (Table 1).

All tumors with high mitotic index were IDC, frequently were high grade, hormone receptor and triple negative and often (24/32; 75%) had pushing tumor borders with zones of necrosis (Figure 1).

Immunohistochemistry for PDL1, MIB1, GATA3, cytokeratins (CK: AE1/3, 18/CAM5.2, 7, 20, 5/6), p63, androgen receptor (AR) and MSI (MLH1, MSH2, MSH6, PMS2) was evaluated in 32 IDC with high mitotic index. Proliferation index ranged from 25-90% (median 55). All had intact MSI markers and were CK20 negative. AR was expressed in ≤10% of tumor cells in 8 (25%). CK5/6 was expressed (≥1%) in 22 (68.8%). PDL1 showed ≥1% positivity in 9 (28.1%) and ≥50% in 2 (6.3%).

All had expression of CK AE1/3 and 18/CAM5.2; however, the case of IDC with neuroendocrine differentiation had CK7 loss and 4 (12.5%) tumors had reduced, ≤10% tumor cell, expression of one (2) or two (2) of these CKs. GATA-3 was negative in 15 (46.9%) and expressed in ≤10% of tumor cells in 2 (6.3%).

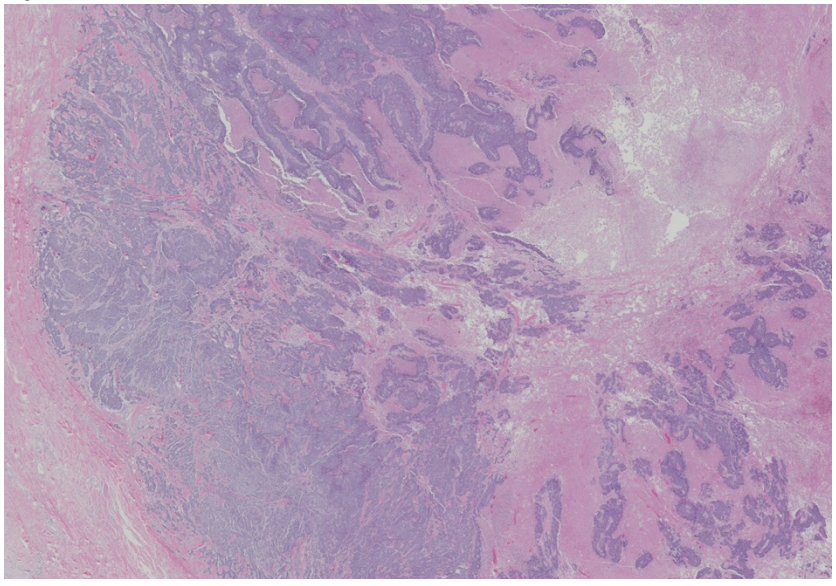
Table 1. Clinical-pathologic Features of Cases with Low-Intermediate and High Mitotic Score Status-Post Neoadjuvant Chemotherapy (NAC).

| | Mitoses Score 1-2 (N=111 tumors from 110 patients) | Mitoses Score 3 (N=32) |
|---|---|---|
| Age (mean (range)) (yrs) | 53.4 (23-83) | 50.6 (29-81) |
| Race (N, %) | | |
| Caucasian | 82 (74.5) | 19 (59.4) |
| African American | 18 (16.4) | 12 (37.5) |
| Hispanic | 4 (3.6) | 1 (3.1) |
| Asian | 4 (3.6) | 0 (0) |
| Other | 2 (1.8) | 0 (0) |
| Menopausal (N, %) | | |
| Pre | 49 (44.5) | 14 (43.8) |
| Post | 60 (54.5) | 18 (56.3) |
| N/A (male) | 1 (0.9) | 0 (0) |
| BMI (N, %) | | |
| <25 | 30 (27.3) | 9 (28.1) |
| 25-29 | 29 (26.4) | 10 (31.3) |
| >30-39 | 42 (38.2) | 13 (40.6) |
| ≥40 | 9 (8.2) | 0 (0) |
| Clinical T Stage (N, %) | | |
| 1 | 18 (16.2) | 1 (3.1) |
| 2 | 61 (55) | 21 (65.6) |
| 3 | 30 (27) | 9 (28.1) |
| NA | 2 (1.8) | 1 (3.1) |
| Positive (Pre-NAC) Lymph Node Status (N, %) | 77 (69.4) | 22 (68.8) |
| Clinical Stage (N, %) | | |
| I | 4 (3.6) | 0 (0) |
| II | 61 (55) | 18 (56.3) |
| III | 46 (41.4) | 14 (43.8) |
| Histology (N, %) | | |
| IDC | 95 (85.6) | 32 (100) |
| ILC | 16 (14.4) | 0 (0) |
| | | |
| Modified Bloom-Richardson Grade (N, %) | | |
| 1 | 17 (15.3) | 0 (0) |
| 2 | 61 (55) | 3 (9.4) |
| 3 | 33 (29.7 | 29 (90.6) |
| Hormone Receptor Status (N, %) | | |
| Positive | 89 (80.2) | 9 (28.1)^ |
| Negative | 22 (19.8) | 23 (71.9) |
| HER2 Status (N, %) | | |
| Positive | 16 (14.4) | 6 (18.8) |
| Negative | 91 (82) | 26 (81.3) |
| Equivocal | 3 (2.7) | 0 (0) |
| Not Available | 1 (0.9) | 0 (0) |
| Triple Negative (N, %) | 16 (14.4) | 20 (62.5) |
| Lumpectomy (N, %) | 37 (33.3) | 9 (28.1)* |
| Mastectomy (N, %) | 74 (66.7) | 24 (75)* |
| Sentinel Lymph Node Excision (N, %) | 43 (38.7) | 14 (43.8) |
| Axillary Lymph Node Dissection (N, %) | 68 (61.3) | 18 (56.3) |
| Radiation Therapy (N, %) | 85 (77.3) | 21 (65.6) |
| Recurrence (N, %) | 18 (16.4) | 17 (53.1) |
| Metastasis (N, %) | 22 (20) | 18 (56.3) |
| Deceased (N, %) | 18 (16.4) | 15 (46.9) |
| Post-NAC (yp) T Stage (N, %) | | |
| 1 | 52 (46.8) | 12 (37.5) |
| 2 | 38 (34.2) | 15 (46.9) |
| 3 | 19 (17.1) | 3 (9.4) |
| 4 | 2 (1.8) | 2 (6.3) |
| Post-NAC Tumor Size (mean, range) (N, %) | 3.1 (0.3-12) | 3 (1-17) |

^One patient was ER (8%) focal positive, PR negative, HER2 negative

* One patient had both lumpectomy and mastectomy

Figure 1 - 109



**Conclusions:** IDCs with high mitotic rate following NAC frequently have a distinct phenotype, with pushing borders and necrosis, high proliferation index, and may show loss of cytokeratin and GATA3 expression. This phenotype can frequently be recognized on biopsy and appears to have poorer response to standard chemotherapy, with high morbidity/mortality.

## 110 Impact of New Prognostic Staging System (AJCC 8th Edition) and Genomic Analysis of Tumors on Disease Outcome in ER positive and HER-2 Negative Breast Cancer

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**Background:** The 8th edition of the American Joint Committee (AJCC) on Cancer Staging Manual recommends the use of new Prognostic Stage (PS) group system for staging breast cancer patients. In addition to the TNM (primary tumor T, regional lymph nodes N, distant metastases M) system, which was traditionally used in the pathologic staging, the new PS incorporates other biologic factors such as tumor grade, proliferation rate, estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2) expression, and gene expression prognostic panels such as Oncotype Dx into the staging system. We aim to evaluate the impact of PS grouping on tumor recurrence and outcome and compare it to the Anatomic Stage (AS) group that has been the standard of care prior to 2018

**Design:** Pathology database between 2006-2012 was retrospectively reviewed for patients with ER positive, HER2 negative invasive mammary carcinomas, and known Oncotype Dx scores, who had surgery as their initial treatment. Patients were assigned a new PS group based on AJCC 8th edition, and compared to their original AS group. Follow-up period ranged from 5 to 11 years. Rates of tumor recurrence/metastases and/or death of disease were determined in group IA patients. Data analysis was performed using Chi-Square test and *p* value of $< 0.05$ was considered significant

**Results:** A total of 392 cases were retrieved. Ninety-six patients (24.5%) were down-staged, only 1 case (0.26%) was upstaged, while the remaining 295 patients (75.3%) remained unchanged, 279 of which remained in stage IA. Of the 96 down-staged patients, 73 (76%) were down-staged to PS Stage IA. Low Oncotype DX score (score <11) caused direct down-staging of 19 cases (26%). At the end of follow-up period, death of disease occurred in 3/73 (4.1%) patients who were down-staged to PS group IA, and 8/279 (2.8%) of the patients who remained in stage 1A died of disease ($p= 0.45$). Disease recurrence and/or distant metastases were observed in 8/73 of patients who were down-staged to group IA (11%), compared to 10 (3.6%) patients in the unchanged IA group ($p=0.007$)

**Conclusions:** Our data shows that patients, who were down-staged to PS group IA using the new AJCC staging criteria, had a higher disease recurrence/metastases rate compared to those who were unchanged. Larger studies with longer follow-up are needed to establish the clinical impact of the new PS grouping system on breast cancer patients, in order to provide better data about the outcome of their disease

## 111 Breast Cancers with HER2/CEP17 Ratio ≥2.0 and an Average HER2 Copy Number < 4.0 per Cell: Clinicopathologic Features and Alternative Chromosome 17 Probe Analysis of 102 Cases from a Single Institution Study

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**Disclosures:** Qianming Bai: None; Shuling Zhou: None; Xiaoyan Zhou: None; Wentao Yang: None

**Background:** The 2013 ASCO/CAP guidelines classified breast cancers with HER2/CEP17 ratio ≥2.0 by FISH as "amplified", inclusive of cases with an average HER2 copy number < 4.0 per cell (Group 2), which was recommended to re-define the HER2 status according to the associated immunohistochemical findings in 2018 ASCO/CAP update. However, because of its ratity and limited clinical data, the clinicopathologic features and efficacy of HER2-targeted therapy in this small subset of cases were still unclear.

**Design:** Breast cancers with HER2 FISH results in the Group 2 category were retrieved from a single institution during 2014-2017. Clinicopathologic features and the efficacy of HER2-targeted therapy were analyzed retrospectively. In addition, several alternative chromosome 17 reference genes, including Smith-Magenis syndrome (SMS), retinoic acid receptor alpha (RARA), and tumor protein 53 (TP53), were used to re-assess HER2 gene status.

**Results:** 102 (1.0%) of 10177 primary and metastatic breast cancers with Group 2 HER2 FISH results were identified. The mean age at diagnosis was 50 years and the majority of cases were histological grade II-III (89%), ER positive (76%), and HER2 IHC 2+ (72%). NO case with HER2 IHC 3+ was observed.

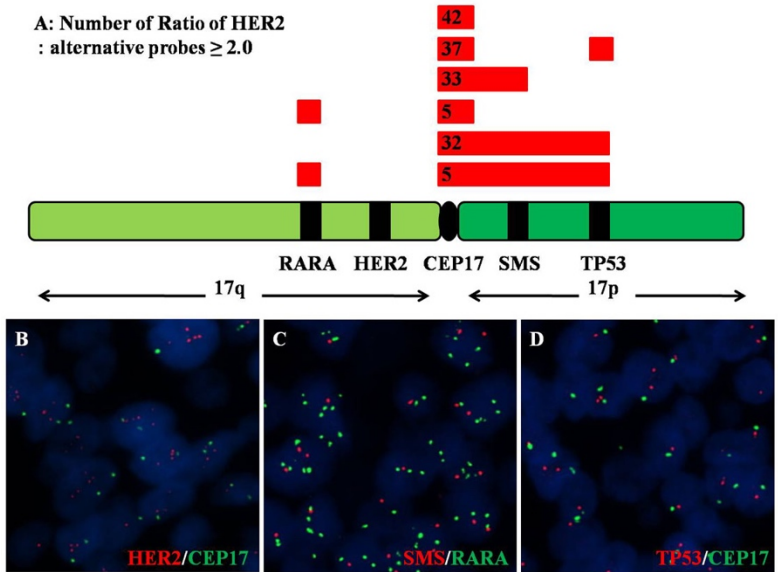
Several alternative chromosome 17 reference gene probes were performed on 42 cases. The number of ratio of HER2 : alternative probes was shown in Fig.1A on the basis of one of the alternative probes and various combinations, respectively. Among these three alternative genes, average RARA copy number was similar with HER2 in the most cases (88.1%), while SMS (78.6%) and TP53 (88.1%) often showed similar copy number with CEP17 (Fig.1B-D).

Of these 42 patients, targeted therapy with trastuzumab was performed on 22 cases with follow-up ranging from 10-56 months (median = 35 months). Compared to other treatment, the patients didn't show a significant benefit from targeted therapy. However, there were fewer patients with progression disease (PD) in the group with the ratio still ≥2.0 by using the alternative probes, although no statistical significance because of limited data (Table 1).

Efficacy of HER2-targeted therapy in breast cancers with the ratio of HER2 / alternative probes ≥2.0 and an average HER2 copy number < 4.0 per cell.

| Alternative probes | Targeted therapy | Alive without progression disease | Progression disease | P value |
|---|---|---|---|---|
| **CEP17** | YES | 18 | 4 | 1.000 |
| | NO | 16 | 4 | |
| **RARA** | YES | 2 | 0 | 1.000 |
| | NO | 2 | 1 | |
| **SMS** | YES | 14 | 2 | 0.656 |
| | NO | 13 | 4 | |
| **TP53** | YES | 16 | 3 | 0.693 |
| | NO | 14 | 4 | |
| **SMS and TP53** | YES | 14 | 2 | 0.654 |
| | NO | 12 | 4 | |
| **SMS and TP53 and RARA** | YES | 2 | 0 | 1.000 |
| | NO | 2 | 1 | |
| **SMS or TP53 or RARA** | YES | 16 | 3 | 1.000 |
| | NO | 15 | 4 | |

Figure 1 - 111




**Conclusions:** Breast cancers with HER2/CEP17 ratio ≥2.0 and an average HER2 copy number < 4.0 per cell were infrequent. Our results indicated that these patients didn't benefit from HER2 targeted therapy, however, incidence of PD decreased in the group with the ratio of HER2 : SMS and/or TP53 still ≥2.0. Thus, such patients still need more attention.

## 112 Effects of Oncogenes on Mammary Stem Cells during Breast Cancer Development in Tg11.5kb-GFP Mice

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**Disclosures:** Lixia Bai: None; Larry Rohrschneider: None; Xueyan Chen: None

**Background:** A novel shorter SH2-containing inositol 5'-phosphatase (SHIP) isoform s-SHIP has been identified specifically in embryonic stem cells and adult hematopoietic stem cells, but not in differentiated cells. Using transgenic mice termed Tg11.5kb-GFP, in which enhanced green fluorescent protein (GFP) expression is driven by the 11.5kb s-SHIP promoter, we demonstrated that $GFP^+$cap cells at puberty and basal alveolar bud cells at pregnancy are proliferating mammary stem cells (MaSCs). We propose that $GFP^+$MaSCs are potentially transformed by some oncogenes that are highly expressed in breast cancers (such as *Wnt* and *ErbB2*) during mammary tumorigenesis.

**Design:** We crossed Tg11.5kb-GFP mice with two breast tumor mouse models: TgMMTV-Wnt1 and TgMMTV-ErbB2. We examined GFP expression in mammary glands at puberty and pregnancy from Tg11.5kb-GFP;MMTV-Wnt1 and Tg11.5kb-GFP;MMTV-ErbB2 mice, characterized $GFP^+$cells by immunofluorescence microscopy and flow cytometry, and analyzed the MaSC activity of $GFP^+$cells by cleared fat pad transplantation.

**Results:** We found that at puberty, GFP expression is turned on in cap cells of both $Wnt1^+$and $ErbB2^+$ mammary tissues. Compared with mammary glands of $GFP^{+/-}Wnt1^{-/-}$littermates at 5 week-old, the percentage of $GFP^+$cap cells in $GFP^{+/-}Wnt1^{+/-}$glands increased more than two-fold. The percentage of $GFP^+$cap cells in $GFP^{+/-}ErbB2^{+/-}$glands was similar to that of $GFP^{+/-}ErbB2^{-/-}$littermate glands. No $GFP^+$epithelial cells were observed in $Wnt1^+$or $ErbB2^+$ adult mammary glands. $Wnt1^+$glands exhibit extensive ductal hyperplasia compared with $Wnt1^-$ littermates, whereas the morphology of $ErbB2^+$glands was similar to that of $ErbB2^-$littermate tissues. Interestingly, we did not find obvious $GFP^+$epithelial cells in $GFP^{+/-}Wnt1^{+/-}$glands at pregnancy. The percentage of $GFP^+$cells in $GFP^{+/-}ErbB2^{+/-}$glands at pregnancy was not significantly different from that of $GFP^{+/-}ErbB2^{-/-}$littermate glands. Immunostaining did not detect milk production in $Wnt1^+$glands on lactation day 0.5, which explains why TgMMTV-$Wnt1^+$female mice cannot nurse their pups after giving birth. Upon transplantation, $GFP^+Wnt1^+$cap cells regenerate hyperplastic mammary epithelial outgrowths.

**Conclusions:** Wnt1 stimulates $GFP^+$MaSC proliferation and ductal hyperplasia at puberty, and inhibits MaSC differentiation for milk generation at pregnancy and lactation. ErbB2 has no impact on $GFP^+$MaSC proliferation and differentiation during development. Our findings suggest that $GFP^+$MaSCs are likely the transformation targets of Wnt1, but not targets of ErbB2.

## 113 Flat Epithelial Atypia Diagnosed on Core Needle Biopsy: Reasons for Exclusion from a Multi-Center Prospective Trial Following Central Pathology Review

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**Disclosures:** Gabrielle Baker: None; Faina Nakhlis: None; David Suster: None; Tari King: None; Stuart Schnitt: None

**Background:** Flat epithelial atypia (FEA) is a clonal proliferation characterized by low-grade, monomorphic cytologic atypia also seen in atypical ductal hyperplasia (ADH) and ductal carcinoma in situ of low nuclear grade (DCIS-LG). However, by definition, FEA lacks the architectural atypia that defines ADH and DCIS-LG (e.g. rigid arcades, cribriforming, micropapillae). Whereas the standard of care for ADH and DCIS-LG diagnosed on CNB consists of excision, the optimal management for FEA only diagnosed on CNB is uncertain. Given the wide range in upgrade rates reported in the literature and the retrospective nature of most studies, a multi-center prospective trial is being conducted to better understand the upgrade rate at surgical excision of FEA diagnosed on CNB. This analysis reports on cases excluded from the study thus far based on central review.

**Design:** The first 44 patients with a diagnosis of FEA on CNB enrolled in a multi-center prospective trial (TBCRC-034) represent the population for this analysis. The primary endpoint of this trial is to evaluate the upgrade rate on surgical excision. Selected slides from the CNB and excision specimens for each case were submitted for central pathology review.

**Results:** Fourteen of 44 cases (32%) were excluded from the study due to a change in diagnosis on central pathology review of the CNB. Of these, 11 (79% of excluded cases, 25% of total) had features diagnostic of ADH in addition to FEA. Two (14% of excluded cases, 4.5% of total) lacked atypia on central review. In both of these cases the alteration misconstrued as FEA was columnar cell change (CCC). Two cases were excluded for other reasons: one case had cytologic atypia greater than what is permitted for a diagnosis of FEA or ADH and one case had a complex sclerosing lesion (CSL). Of note, one of the aforementioned cases was excluded due to both lack of atypia and presence of a CSL.

**Conclusions:** The diagnosis of FEA on CNB may be challenging and in this preliminary study the CNB diagnosis was changed in 32% of cases. Underdiagnosis of ADH as well as overinterpretation of non-atypical proliferations was observed. Of note, the original pathology report noted the presence of architectural atypia (i.e. the definition of ADH) in 3 of the 11 cases excluded due to the presence of ADH on CNB. These findings underscore the importance of adhering to established diagnostic criteria, namely that the presence of architectural atypia by definition precludes a diagnosis of FEA.

## 114 Nipple Piercing-Associated Infections: Case Series with Review of the Literature and an Association with Granulomatous Mastitis

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**Disclosures:** Gabrielle Baker: None

**Background:** Nipple piercing (NP) has been cited as a risk factor for the development of breast abscess; however, the prevalence of NP and the frequency of NP-associated infection (NPAI) are unknown. The relevant literature consists predominantly of case reports that lack adequate histologic description. The purpose of this study is to evaluate the microorganisms associated with (w) NPAI and to assess the pattern of associated inflammatory infiltrate.

**Design:** A ten-year single-institution review was performed to identify cases of NPAI; associated microbiology results were identified and the corresponding slides were evaluated. The literature was reviewed for reports in which infection was associated w NP.

**Results:** 5 cases of NPAI were identified on institutional review (age range 19-37yo, mean 30yo, median 33yo; all female). Bacterial culture was performed in 4 of 5 cases and all were positive: 2 w coagulase negative *Staphylococci*, 1 w *Corynebacterium amycolatum/xerosis* (*Propionibacterium acnes* and *Haemophilus parainfluenzae* also present), and 1 w rare gram positive cocci not otherwise specified (NOS). Histologic evaluation demonstrated granulomatous inflammation in all 5 cases; 4 had suppurative granulomatous mastitis w or w/out cystic spaces.

Literature review identified 23 additional cases of NPAI (age range 15-60yo, median and mean 28yo; 20 female, 3 male). Culture was performed in 16 cases and 15 were positive (6 polymicrobial; one additional case had positive gram stain only). The organisms identified were *Staphylococcus spp* (n=6), atypical mycobacteria (n=5), *Streptococcus spp* (n=4), *Prevotella spp* (n=2), *Actinomyces* spp (n=1), *Gordonia terrae* (n=1), *Nocardia spp* (n=1), *Peptostreptococcus spp* (n=1), diphtheroids NOS (n=1), and gram positive bacilli NOS (n=1). Histologic description was available for 5 cases: all had granulomatous inflammation.

**Conclusions:** All cases in the present series and at least 5 cases in the literature had granulomatous inflammation. The presence of suppurative granulomatous inflammation w/ or w/out cystic spaces is characteristic of Cystic Neutrophilic Granulomatous Mastitis, a disease associated with coryneform bacteria and only reported in one prior case of NPAI. To the author's knowledge, this represents the largest case series of NPAI in the pathology literature and provides the greatest information regarding histology of NPAI. Additionally, the spectrum of NPAI-associated microorganisms is expanded.

## 115 Expression Of Obesity Related Genes in Women with Breast Cancer (BC)

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**Disclosures:** Sudeshna Bandyopadhyay: None; MHD Fayez Daaboul: None; Hany Deirawan: None; Neeraja Yerrapotu: None; Ibrahim Tsolakian: None; Karim Dirani: None; Cristina Mitrea: None; Aliccia Bollig-Fischer: None; Rouba Ali-Fehmi: None

**Background:** For estrogen receptor (ERα) positive, hormone-dependent breast cancer in post-menopausal patients the association between obesity and cancer recurrence and reduced survival was recognized early.

Recent studies report that for younger, pre-menopausal patients, obesity is a risk factor for triple negative breast cancer (TNBC) diagnosis and worse outcomes. Details surrounding the molecular mechanisms linking obesity and breast cancer remain to be resolved. We hypothesized that the influence of obesity on tumor molecular biology plays a role in obese breast cancer patients, irrespective of subtype.We studied the tumor transcriptome of obese and non obese women with breast carcinoma for new insights.

**Design:** Using the Illumina HiSeq2500 and TruSeq RNA Exome library prep kit, we performed RNA-sequencing analysis to measure gene transcript levels in RNA isolated from FFPE tumors. Cufflink and Cuffdiff software tools were used in data analysis comparing expression levels in tumors from obese and non-obese breast cancer patients (Figure **1**). The analysis combined ER positive and triple negative breast cancers in non-obese and obese categories. (Table **1**)

**Results:** Comparing gene expression levels in tumors from obese patients to tumors from non-obese patients identified 665 significantly changed genes ($p<0.01$, fold-change > 2) and a significant enrichment of an array of biological functions associated with the obese phenotype was seen, with methylation being the top enriched biological function along with other DNA modifying functions (**Fig. 2A**). In addition, we observed a significant increase and greater diversity in the expression of variant transcripts indicating that alternative mRNA splicing may be tied to obesity and may be important for both cancer subtypes (**Fig. 2B**).

Table 1: ER positive and triple negative patients in obese vs non-obese patients

| | BMI 21-30 | BMI 31-41 |
|---|---|---|
| **ER positive** | **27** | **18** |
| **Triple Negative** | **1** | **6** |

Figure 1-115

Figure 1. Enrichment analysis for gene-protein interactions using the Enrichr tool and huMAP data base applied to the significant differentially expressed genes (DE, n=665, p<0.01; fold-change>2) that resulted from comparing RNA-seq data associated with breast tumors from patients with BMI greater than 30 relative to patients with BMI less than 30. The top ranked results suggest that, based on genes identified in RNA-seq analysis, the proteins listed in the left column may be activated in the tumors from obese patients. Enrichr (combined) score is based on a corrected pvalue. Future work will explore if results here are indicating how multiple mRNA splicing factors, in addition to SRSF2 (already being investigated by us), may be effecting the greater diversity of isoform expression in tumors from obese women (Fig. 1B).

| Symbol | Name | Function | pvalue | Enrichr Score |
|---|---|---|---|---|
| EIF4A3 | Eukaryotic translation initiation factor 4A3 | mRNA nonsense mediated decay | 0.001159 | 11.0674 |
| CDK11A | Cyclin-dependent kinase 11A | serine/threonine kinase, mRNA splicing | 0.020745 | 5.682801 |
| SRSF9 | Serine/arginine-rich splicing factor 9 | alternative mRNA splicing | 0.035219 | 6.390531 |
| SRSF10 | Serine/arginine-rich splicing factor 10 | alternative mRNA splicing | 0.044094 | 5.683844 |
| THRAP3 | Thyroid hormone receptor associated protein 3 | RNA processing | 0.058435 | 5.305264 |

Figure 2-115

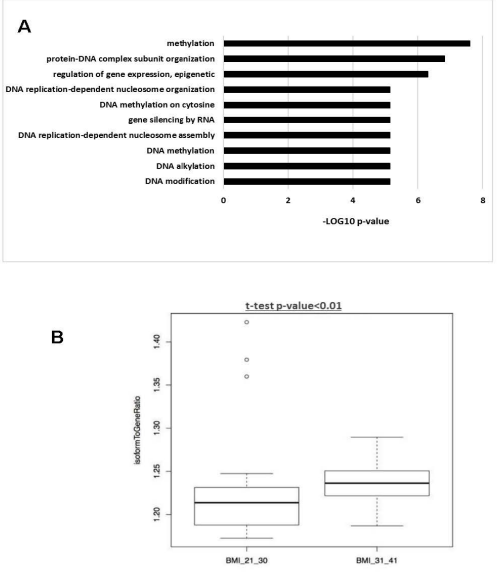


**Fig. 2**. RNA-sequencing analysis of breast tumors from non-obese and obese patients. **(A)** Differential expression analysis was performed using RNA-sequencing data from tumor groups, iteratively partitioned to test all possible BMI thresholds. The most comprehensive, significantly changed gene set was observed when we compared groups of cancer specimens from patients with BMI ≤ 28 and BMI ≥ 30. Further analysis of the 665 significantly changed genes (p<0.01, fold-change > 2) using IPathway Guide tools demonstrated biological function over-enrichment, the top 10 significant categories are shown. **(B)** Analysis of mRNA variant expression using RNA-sequencing data from patient breast cancer samples. For each sample, the number of variant isoforms mapped in each RNA sequencing data set were normalized to all genes mapped in that data set. A significant global difference was observed for cancer samples from patients with BMI 21-30 compared to values from patients with BMI 31-41, where the numbers of variants expressed increased with increasing BMI.

**Conclusions:** Rising obesity rates threaten to further increase the burden of obesity-linked cancers especially breast cancer. We show a significant alteration in the transcriptome of obese women with breast carcinoma compared to non-obese. This reinforces the relevance of our research efforts to understand the molecular biology driving obesity related cancers.

## 116 Comparison of Next-Generation Sequencing and Immunohistochemistry for Assessment of MCL-1 Amplification and Protein Overexpression in Triple Negative Breast Carcinoma: An Emerging Biomarker

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**Disclosures:** Jordan Baum: None; Hung Tran: None; Hanna Rennert: None; Helen Fernandes: None; Paula Ginter: None

**Background:** MCL-1, a member of the BCL2 pro-survival protein family, confers cell survival through inhibition of apoptosis. This locus is frequently amplified in breast cancer, particularly triple negative breast carcinoma (TNBC). MCL-1 is an emerging biomarker, however, protocols for its assessment have not yet been well established. Studies regarding patient prognosis have largely focused on MCL-1 protein overexpression by immunohistochemistry (IHC), while clinical trials are increasingly relying on next-generation sequencing (NGS) to identify patients with genomic alterations for “basket trails”. Our aim was to investigate the correlation between gene amplification with protein overexpression in TNBC.

**Design:** 30 TNBC excisions were identified from 2004 to 2015 in our archives. FFPE tissue blocks were cored and extracted DNA was subjected to targeted NGS using the Oncomine Comprehensive Panel v2 and Ion PGM platform. Data analysis was performed using the Ion Reporter v5.0. The minimum 5% confidence interval threshold for calling an amplification was set at ≥ 4.0 copy number score. MCL-1 IHC was performed and assessed by the standard semi-quantitative Histoscore method (H-score, 0-300) by two pathologists blinded to the NGS results.

**Results:** 10 of the 30 cases showed *MCL1* amplification by NGS. Copy number scores in the amplified cases were 4.1-9.8. The mean H-score for the amplified cases (259 ± 36.6, 95% CI: 236.2-281.8) was significantly higher than the non-amplified cases (137.2 ± 59.7, 95% CI: 111-163.4, $p$<0.001). H-scores for NGS-amplified cases were 195-295 with 9/10 cases >200, non-amplified were 5-240 with only 3/20 cases >200. H-scores in the range of 195-240 were both amplified (n=4) and non-amplified (n=3), while cases below and above this range were exclusively non-amplified or amplified, respectively. While all NGS-amplified cases showed relatively high H-scores, there was no positive correlation between H-score and copy number score values in the NGS-amplified cases ($R^2$=0.001).

**Conclusions:** Moderate to strong IHC was seen in all cases of *MCL1* amplification by NGS. A small proportion of cases, however, showed protein overexpression without amplification, indicating alternative mechanisms. While NGS identifies DNA amplified cases, this small proportion of IHC overexpressed cases would be missed using NGS. As multiple studies have indicated that MCL-1 IHC protein overexpression is a poor prognostic factor, relying solely on NGS for prognostic information may fall short.

## 117 MCL-1 Protein Overexpression Correlates with Adverse Clinicopathologic Features in Triple Negative Breast Carcinoma

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**Disclosures:** Jordan Baum: None; Zhengming Chen: None; Sandra Shin: None; Paula Ginter: None

**Background:** Triple negative breast carcinoma (TNBC) is an aggressive form of breast cancer. MCL-1 (Myeloid cell leukemia 1), a member of the pro-survival protein family, is frequently amplified in breast cancer. Some studies have shown that MCL-1 protein expression correlates with clinicopathologic features in breast cancer while others have failed to show such correlations. Our study aimed to assess the correlation between MCL-1 expression with clinicopathologic features and prognosis in TNBC.

**Design:** Anti-MCL-1 immunohistochemistry (IHC) was performed on tissue microarrays from 222 cases of primary TNBC from 2000-2015. Staining was assessed by Histoscore (H-score) technique. Two H-score cutoffs to designate protein overexpression were used for analysis (140 “low-positive [LP]” and 195 “high-positive [HP]”). Clinicopathologic features were compared using *t*-test and Fisher's exact test. Survival data was available for 139 cases in the cohort. Kaplan-Meier estimator and log-rank test were for statistical significance between breast cancer specific overall survival (BCS-OS) and disease-free survival (DFS).

**Results:** 56 cases showed LP and 25 cases showed HP MCL-1 staining. LP cases were significantly associated with younger age than MCL-1 non-overexpressed (NO) (mean: 52 vs 60, P<0.05); however, HP cases were not significantly different than NO (mean: 56 vs 58, P=0.5). LP was significantly associated with and HP trended toward overall higher pT category (P<0.05 and P=0.11, respectively). Both HP and LP significantly associated with higher pT category when comparing pT1 vs pT2-4 (P<0.05). MCL-1 NO cases were significantly more likely to be very early stage (stage 1 vs stage 2-4) than both LP and HP cases (P<0.05). LP and HP cases trended toward higher tumor

grade with 91% of LP grade 3 vs 80% of NO cases (P=0.07) and 96% of HP grade 3 vs 81% NO cases (P=0.09). There was no difference in pN category, BCS-OS, or DFS.

**Conclusions:** To our knowledge, this study represents the largest cohort of primary TNBC to date to be evaluated for MCL-1 overexpression by IHC and is the first study to evaluate clinicopathologic features at both LP and HP cutoffs. While MCL-1 overexpression was associated with adverse clinicopathologic features, no difference in BCS-OS nor DFS was observed in this cohort, likely due to the low number of events. MCL-1 overexpression is a promising biomarker in TNBC, however additional studies are necessary to determine the prognostic significance in TNBC.

## 118 Evaluating the ability of multiphoton laser-scanning microscopy to predict triple negative breast carcinoma (TNBC) response to neo-adjuvant therapy

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**Disclosures:** Monisha Bhanote: None; Danielle Desa: None; Edward Brown: None; Ioana Moisini: None; David Hicks: None; Bradley Turner: None

**Background:** The extracellular matrix (including fibrillar collagen) has been suggested to play a role in tumor migration and metastatic disease. Second Harmonic Generation (SHG) is a unique imaging methodology that quantitatively evaluates an intrinsic optical signature produced by fibrillar collagen. Burke et al. (BMC Cancer.15 [2015]) published that the average forward to backward-light scattering ratio (F/B) produced by this optical signature is an independent prognostic indicator of metastasis free breast cancer survival. In Her-2 +patients status post neoadjuvant chemotherapy, we have observed that lower F/B measurements at the tumor-stromal interface are associated with a higher Residual Cancer Burden (RCB) class (2018 San Antonio Breast Cancer Symposium presentation), suggesting that F/B may be useful for predicting pathologic response in Her-2 +patients. The goal of the current study was to evaluate F/B in the tumor and extracellular matrix, and to correlate F/B with the RCB class, in TNBC patients status post neoadjuvant chemotherapy.

**Design:** 28 TNBC patients status post neoadjuvant chemotherapy with surgical excision between 2008 and 2018 were identified from the database at our institution. 10 had a pathologic complete response (pCR/ RCB class 0). 18 did not have a pCR (non-pCR/RCB class 2-3). We used SHG imaging to determine the average F/B in the tumor bulk and tumor-stromal interface. We compared the F/B in the tumor bulk to the tumor-stromal interface, and correlated F/B with the RCB class.

**Results:** Figures 1 and 2 show a histologic section and the corresponding SHG image. The F/B at the tumor-stromal interface was significantly higher than the F/B in the tumor bulk in all RCB classes ($p < 0.001$). Patients with a RCB class 2-3 (non-pCR) tended to have a *higher* F/B within the tumor bulk, with a *lower* F/B at the tumor-stromal interface, compared to patients with a RCB class 0 (pCR), although not reaching statistical significance ($p = 0.42$ and 0.95, respectively).

Average F/B and pathologic response

| **Pathologic response (n)** | **Average F/B** | |
|---|---|---|
| | Tumor Bulk | Tumor-stromal interface |
| pCR (10) | 6.6 | 10.59 |
| non-pCR (18) | 7.7 | 10.12 |

Figure 1 - 118

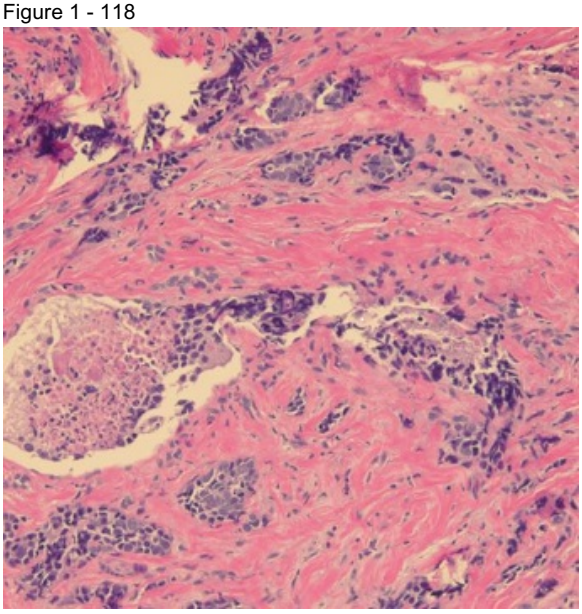
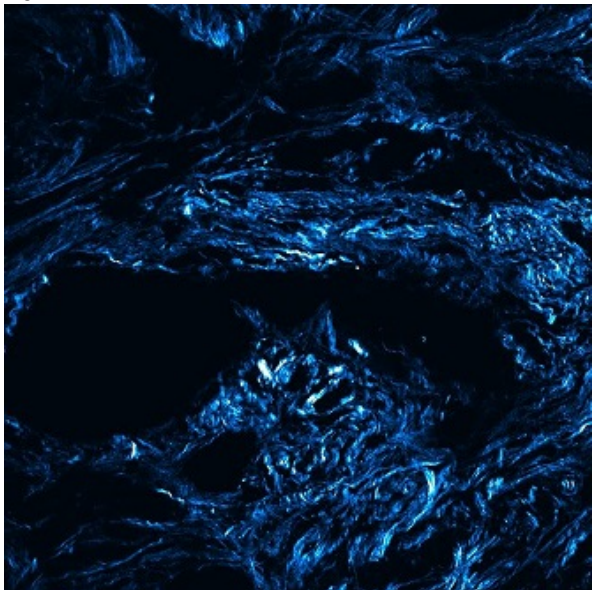


Figure 2 - 118



**Conclusions:** Our results are consistent with previous data suggesting an inverse association of F/B with RCB class at the tumor-stromal interface. Our data also suggests that the opposite relationship exists between F/B and RCB class within the tumor bulk, and that there is F/B heterogeneity between the tumor bulk and the tumor-stromal interface. F/B may be helpful in predicting pathologic response in TNBC patients status post neoadjuvant chemotherapy; however, our results need to be further qualified in a larger population.

## 119 Can Deep Learning Reduce Cost and Turnaround Time? A Fine-Tuned InceptionV3 Convolutional Neural Network Architecture Accurately Distinguishes DCIS from LCIS

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**Disclosures:** Devin Broadwater: None; Andrew Walls: None; Nathaniel Smith: None

**Background:** Use of Convolutional Neural Networks (CNNs) in medicine, particularly radiology and pathology, has steadily increased in recent years. However, utilization of a CNN to reduce or replace immunohistochemical (IHC) staining in diagnostic pathology has been minimal. Here, we demonstrate that fine-tuning of the InceptionV3 CNN architecture accurately and confidently distinguishes between morphologically-difficult cases of ductal and lobular carcinoma in-situ (DCIS/LCIS).

**Design:** A total of 1,522 H&E images of DCIS (636) and LCIS (886) were acquired via a 12 megapixel cell phone camera from 64 archived breast cases that were previously stained with e-cadherin IHC. Correct categorization of the lesions was confirmed through review by an expert breast pathologist in conjunction with e-cadherin IHC. The images were randomly partitioned into 1,209 training (80%) and 313 test images (20%). The training set was used to fine-tune a customized fully-connected (FC) layer on top of the InceptionV3 CNN architecture utilizing pre-trained ImageNet convolutional layer weights. The customized FC layer contained a single 256-unit hidden layer and 2 final output units with softmax activation to output a probability distribution over the two classes.

**Results:** The CNN showed 89% accuracy overall, defined as the model outputting its maximum probability prediction on the true class. Of 313 test images, 258 (82.4%) had a predicted class probability of > 0.8 (“Confident Prediction”) while 55 (17.6%) had a probability of 0.5 to 0.8 (“Equivocal Prediction”). The mean maximum predicted class probability averaged over the entire test set was 0.851 with a standard deviation of 0.241. The diagnostic specificities with “confident” and “equivocal” predictions were 95% and 58.2%, respectively. Hypothetical IHC utilization with only “equivocal” predictions results in 82.4% less e-cadherin IHC usage overall, **Figure 1**. Additionally, 82 cumulative days of turnaround time and $2127.72 worth of reagents would be saved per 100 cases of suspected LCIS. **Figure 2** demonstrates the predicted class probability for various cases of DCIS and LCIS.

Figure 1-119

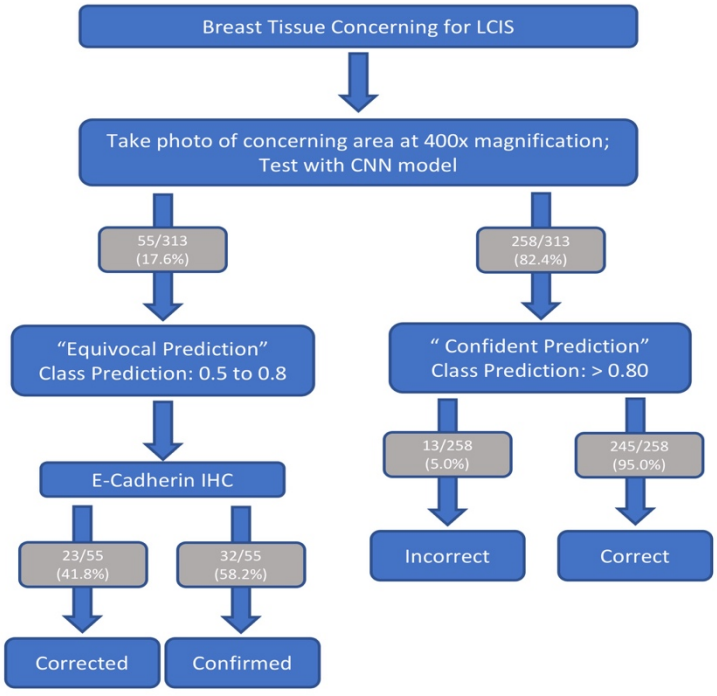
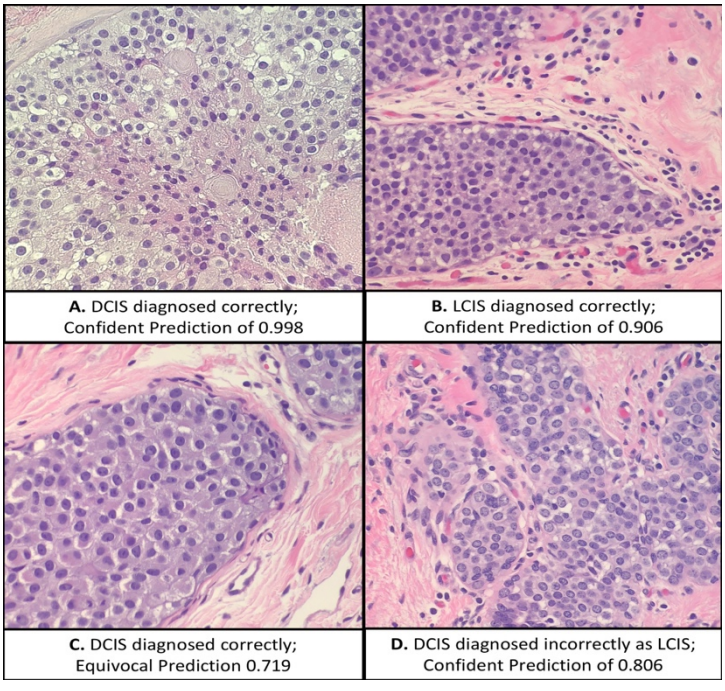



Figure 2-119




**Conclusions:** Fine-tuning the InceptionV3 architecture provides a remarkably accurate and confident CNN model to distinguish LCIS from DCIS. In addition to providing rapid turnaround time from easily obtainable images, total cost per case can be reduced. This research highlights the novel approach of using a CNN to replace or improve IHC staining algorithms for diagnostic purposes.

## 120 The Role of CD73 in Predicting the Pathological Response to Neoadjuvant Treatment in Triple Negative Breast Cancer (TNBC)

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**Disclosures:** Bruna Cerbelli: None; Annalinda Pisano: None; Maria Gemma Pignataro: None; Leopoldo Costarelli: None; Domenico Campagna: None

**Background:** The role of immune system in the tumor surveillance and escape has been long debated. Tumor infiltrating lymphocytes (TILs) and PD-L1 seem to predict pathological complete response (pCR) after neoadjuvant chemotherapy (NAC) in breast cancer. CD73 promotes tumor immune escape through the production of adenosine and has been proposed as a novel biomarker in TNBC, however its role in patient's survival is still unclear. We aimed to investigate the role of CD73, PD-L1 and TILs in predicting pCR in TNBC.

**Design:** Between January 2013 and June 2017, 61 patients with TNBC received standard NAC at our Institutions. We performed immunohistochemistry for PD-L1, CD73, CD20, CD3, CD4, CD8, CD68 and N-CAM on pre-NAC biopsies. The expression of CD73 and PD-L1 by tumor cells was evaluated both quantitatively (percentage of positive cells) and qualitatively (staining intensity); the percentage of TILs was also calculated. Statistical analysis was performed with Mann Whitney test, univariate, and multivariate logistic regression models.

**Results:** Patient's median age was 49 years. In 96.7% the diagnosis was ductal carcinoma NST, G3. The pre-NAC clinical stage was: cT1:13.1%, cT2:75.4%, cT3:4.9%, cT4:6.6%. In 52,5% cases there was nodal disease at presentation. Twenty-three patients (38%) showed pCR. On histology the median expression of CD73 on tumor cells was 40%. In 29 cases (48%) the percentage of positive cells was below and in 32 cases (52%) over this value ("low and "high" CD73). Five out of 61 cases presented PD-L1 expression higher than 25%, a cut-off value that significantly predicts the pCR according to our previous results. In 37,7% of tumors (23/61) the percentage of TILs was more than 40% (high TILs) with predominance of CD8+ subset. PCR was achieved in 48% of "low CD73" tumors (14/29) and 28% of "high CD73" tumors (9/32). Four out of 5 tumors (80%) with high PD-L1 expression achieved pCR; interestingly all these cases belong to the "low CD73" group. Univariate analysis showed a significant association between pCR and both CD73 ($p=0.011$) (expressed as continuous variable) and PD-L1 ($p=0.035$). At multivariate analysis a significant association was found only with CD73 expression ($p=0.027$). No association was found with the percentage of TILs.

**Conclusions:** Low CD73 expression is associated with pCR in TNBC. These preliminary results suggest the possibility of using CD73 inhibitor plus anti-PD-1 in those patients with high CD73 expression.

## 121 Correlation of Clinicopathological features and immunohistochemical markers with molecular subtypes of Triple-Negative Breast Cancer

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**Disclosures:** Satyapal Chahar: None; Aparna Harbhajanka: None

**Background:** Triple-negative breast cancer (TNBC) has the lowest 5-year survival rate of invasive breast carcinomas, and currently there are no approved targeted therapies for this aggressive form of the disease. There is limited literature on clinical relevance and biological markers of the 7-subtypes reported by Lehmann and Bauer et al. The aim was to study the correlation of clinicopathological features and immunohistochemical markers with molecular subtypes of Triple-Negative Breast Cancer.

**Design:** Tissue microarrays of early-stage TNBC were evaluated for biological markers by IHC in 48 cases. The immunohistochemical markers were correlated with clinical and pathologic features such as age, grade, and stage. Gene expression was analyzed on RNA extracted from FFPE specimens with Affymetrix 2.0 HTA. Pietenpol TNBC molecular subtypes were calculated using the online TNBC type tool. Correlation of clinicopathological features and immunohistochemical markers with molecular subtypes of Triple-Negative Breast Cancer were also assessed.

**Results:** The study group was classified by the TNBC subtype analysis tool according to the gene profiling as follows: 21.7% basal like-1( BL1), 17.4% mesenchymal stem cell (MSL) and unstable(UNS) each, 13% immunomodulatory(IM), 10.9% luminal androgen receptor(LAR) and BL2 each and 8.75% mesenchymal (M) subtypes. Morphologically there were 3 metaplastic carcinoma (1 IM, 1 LAR, 1 UNS each), 2 medullary(1 BL1, 1 IM), 1 lobular(MSL), 1 apocrine(LAR), 1 clear cell type (BL1) and others were classified as invasive ducal carcinoma. BL1 show significant higher vimentin expression and lower GCDFP15 and AR expression compared to other molecular subtypes (p-value 0.049, 0.02 and 0.035 respectively). LAR showed significant higher MUC1, AR, GATA-3 and GCDFP15 expression (p-value 0.036, 0.049,

0.077 and 0.07 respectively). UNS show significant higher EGFR expression and lower MUC1 expression (p-value 0.05, 0.09 respectively). IM show significant higher MUC1 (cytoplasmic and membranous pattern) and PDL1 expression (p=0.024, and 0.035 respectively). MSL show significant higher CK5/6 and lower GATA3 expression (p=0.01 and 0.036 respectively). BL2 show significant higher grade and LVI (p= 0.018 and 0.015)

**Conclusions:** Although TNBC is a heterogeneous disease there are significant biological differences in each molecular subtypes based on clinicopathological features and immunohistochemical markers which can be used to further stratify these patients for prognosis and targeted therapy.

## 122 High Expression of UTX Indicates Poor Prognosis in Patients with Luminal Breast Cancer, and is Correlated with MMP-11 Expression

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**Disclosures:**; Kyungseek Chang: None; Dong-Hoon Kim: None; Kyueng-Whan Min: None; Seoung Wan Chae: None; Gi Jeong Kim: None; Sung-Im Do: None

**Background:** Ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX) is a histone H3K27me2/3 demethylase involved in the epigenetic regulation of various genes. A previous study using a mouse xenograft model revealed that UTX knockdown is associated with downregulated expression of matrix metalloproteinase-11 (MMP-11), which enhances the invasiveness of tumor cells. Here, we investigated whether increased expression of UTX is correlated with increased MMP-11 expression and other clinical outcomes in patients with breast cancer.

**Design:** We investigated 224 cases of surgically resected breast cancer from Kangbuk Samsung Medical Center between 2000 and 2005. Nuclear UTX and cytoplasmic MMP-11 expression were assessed using immunohistochemistry of tumor tissue microarray specimens. The relationships between the expression of UTX, MMP-11, and patients' outcomes were analyzed using the Kaplan–Meier method and the Cox proportional hazard model.

**Results:** Using a cut-off value of 5 %, 42 patients (18.8 %) were classified as UTX-positive. UTX expression was significantly associated with high histological grade (p=0.029), lymphatic invasion (p=0.009), vascular invasion (p=0.046) and tumoral expression of MMP-11 (p=0.013). Survival analysis revealed that patients with UTX expression had a poorer overall survival (OS) rate (p=0.010) as well as diminished disease-free survival (DFS) rate (p=0.001). The prognostic power of UTX expression was more significant in patients with luminal-type breast cancer (p=0.027, OS; p=0.008, DFS).

**TABLE 1** Univariate and multivariate Cox regression analyses of UTX expression levels and overall survival.

| | Univariate analysis[a] | | | Multivariate analysis[b] | | |
|---|---|---|---|---|---|---|
| | Hazard ratio | 95 % CI | *p* value | Hazard ratio | 95 % CI | *p* value |
| UTX expression (negative vs. positive) | 2.048 | 1.172–3.578 | **0.012** | 1.476 | 0.822–2.653 | 0.193 |
| T stage (1 or 2 vs. 3) | 4.793 | 2.474–9.285 | **<0.001** | 4.107 | 2.076–8.125 | **<0.001** |
| N stage (1 or 2 vs. 3) | 3.742 | 2.117–6.614 | **<0.001** | 2.540 | 1.353–4.796 | **0.004** |
| Histologic grade (1 or 2 vs. 3) | 3.340 | 1.941–5.748 | **<0.001** | 1.178 | 0.583–2.381 | 0.649 |
| Lymphatic invasion (absence vs. presence) | 3.223 | 1.765–5.884 | **<0.001** | 1.669 | 0.858–3.246 | 0.131 |
| Vascular invasion (absence vs. presence) | 10.545 | 5.572–19.957 | **<0.001** | 6.661 | 3.278–13.536 | **<0.001** |
| Perineural invasion (absence vs. presence) | 3.041 | 1.722–5.369 | **<0.001** | 1.160 | 0.457–2.948 | 0.754 |
| ER/PR status (negative vs. positive) | 2.726 | 1.628–4.566 | **<0.001** | 2.463 | 1.455–4.171 | **0.001** |

UTX, ubiquitously transcribed tetratricopeptide repeat, X chromosome; ER, estrogen receptor; PR, progesterone receptor.
CI, confidence interval
[a]log rank test [b]Cox proportional hazard model p<0.050 is shown in bold

Figure 1-122

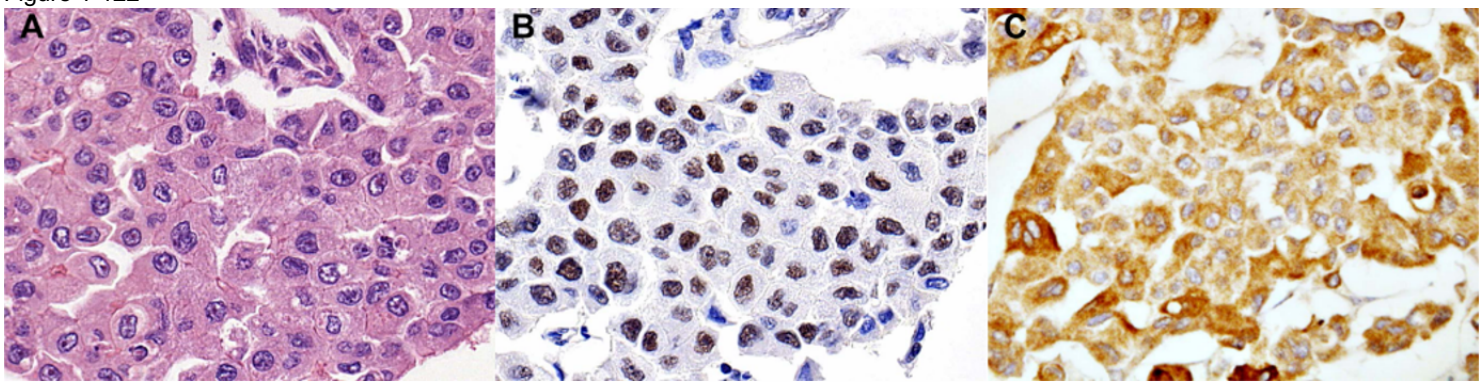


**FIG. 1** Representative case (a) exhibiting nuclear UTX expression (b) and cytoplasmic MMP-11 expression (c). (Original magnification, ×400).

Figure 2-122

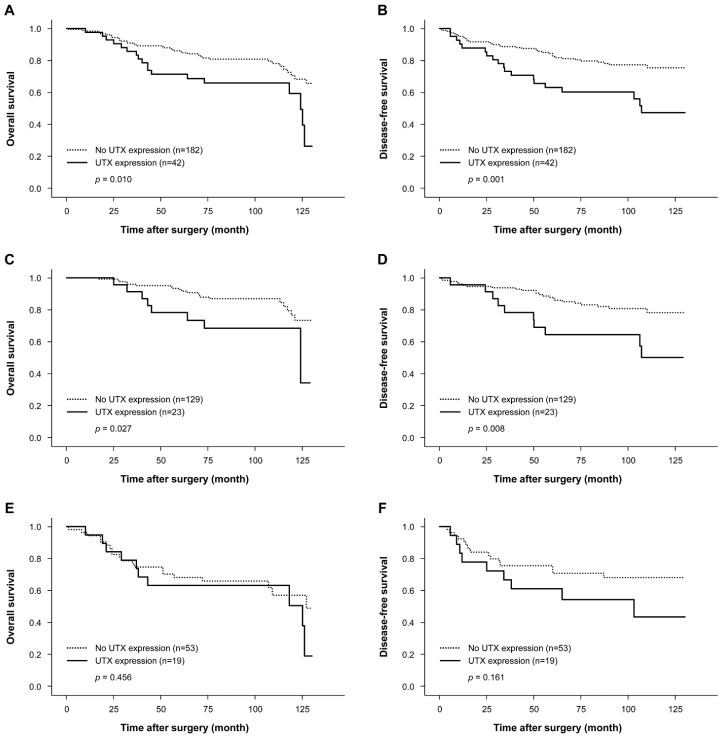


**FIG. 2** Kaplan-Meier plots for overall survival (a, c, e) and disease-free survival (b, d, f) in breast cancers according to UTX expression (log-rank test). Kaplan-Meier plots for UTX expression in all 224 patients (a, b); Kaplan-Meier plots for UTX expression in patients with luminal type breast cancer (c, d); Kaplan-Meier plots for UTX expression in patients with non-luminal type breast cancer (e, f).

**Conclusions:** UTX expression may be useful to predict outcomes in patients with luminal breast cancer. Validation of UTX can provide further prognostic information beyond traditional indicators, and represents a potential therapeutic target for breast cancer.

## 123 Lymphoid Enhancer Binding Factor 1 (LEF1) as a Biomarker for Wnt/Beta-Catenin Pathway Activation in the Biological Progression of Phyllodes Tumors

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**Disclosures:** Po-Han Chen: None; Veerle Bossuyt: None; Emily Reisenbichler: None

**Background:** Phyllodes tumors (PT) are rare epithelial-mesenchymal tumors of the breast with malignant potential. While Wnt/?-catenin has been implicated in the pathogenesis of mammary fibroepithelial lesions, its role in the biological progression of PT remains unclear.

Here, we evaluated the nuclear expression of Lymphoid Enhancer Binding Factor 1 (LEF1), a transcription factor downstream of Wnt/?-catenin signaling, to explore the role of ?-catenin pathway activation in the progression of PT.

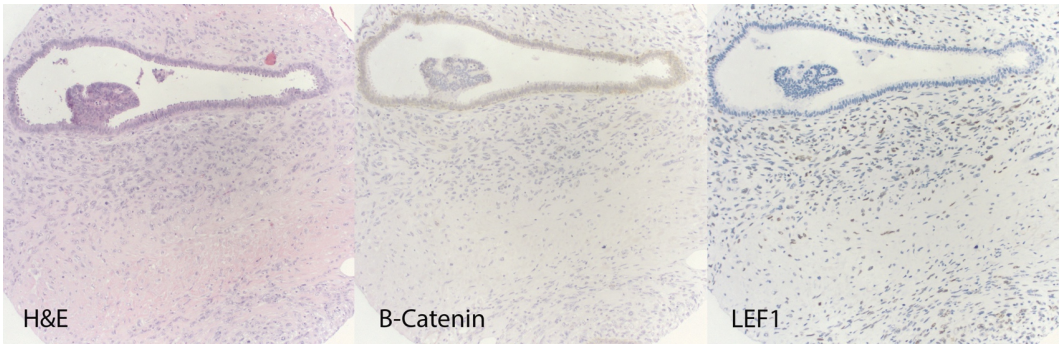
**Design:** A retrospective review of the electronic database was performed to identify excised fibroepithelial lesions of the breast. Cases were reviewed, blinded to the original diagnosis, and classified according to WHO criteria. Cases with a different classification rendered from the original diagnosis were reviewed by a second pathologist and a consensus reached. A tissue microarray (TMA) was made with 2 representative 1 mm cores from each case, composed of 24 benign lesions (including fibroadenomas, cellular and juvenile, and benign PT), 10 borderline (including one periductal stromal tumor) and 8 malignant (including one metastatic phyllodes) tumors. ?-catenin and LEF1 immunohistochemistry (IHC) was performed on the TMA and staining evaluated by H-score (intensity score 0-3 x % cells staining=0-300).

**Results:** Stromal cells expressed LEF1 in 100% (16/16 TMA cores) of malignant, 75% (15/20) borderline, and 27% (13/48) benign tumors with increasing mean H-score seen with increasing tumor grade (Table). Malignant PTs showed significantly higher LEF1 expression than lower grade tumors (p<0.0001). Benign tumors more frequently expressed membranous ?-catenin in the epithelial component than borderline and malignant PTs (70% versus 60% and 40%) with significantly higher H-scores in benign tumors (p=0.004); conversely, stromal nuclear ?-catenin expression was seen in more malignant than borderline and benign tumors (40% versus 25% and 23%). Nine malignant PT cores without stromal nuclear ?-catenin demonstrated strong LEF1 expression (Figure 1).

**Table: Comparison of ?-Catenin and LEF1 Expression in Fibroepithelial Lesions of the Breast**

| | Benign (n=46) | Borderline (n=20) | Malignant (n=16) |
|---|---|---|---|
| **?-Catenin** | | | |
| Mean Epithelial Membranous H-score (range) | 41.1 (0-210) | 15.0 (0-120) | 10.9 (0-90) |
| Mean Stromal Nuclear H-score (range) | 1.24 (0-10) | 1.16 (0-10) | 11.2 (0-120) |
| **LEF1** | | | |
| Mean Stromal Nuclear H-score (range) | 1.54 (0-20) | 6.65 (0-20) | 24.9 (1-150) |

Figure 1-123




**Conclusions:** Both LEF1 and ?-catenin show nuclear expression in many borderline/malignant PTs. This suggests a biological progression of Wnt/?-catenin pathway activation in the stromal component from benign to malignant tumors. Compared to ?-catenin, stromal LEF1 expression presents as a more sensitive marker for high grade PTs and may serve as a new biomarker for separating benign from borderline/malignant tumors.

## 124 Correlation Between Oncotype DS Recurrent Score and Magee Equations Recurrent Score in Canadian Cohort

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**Disclosures:** Lina Chen: None; Sonal Varma: None

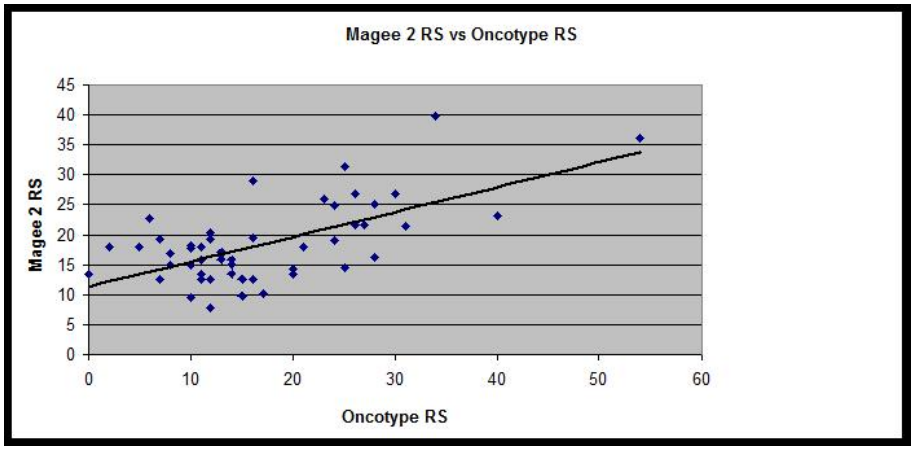
**Background:** Breast cancer is the most common cancer in women and the second most common cause of cancer death in women in North America. Oncotype DX is accepted for clinical use in North America in risk stratifying ER positive breast cancer patients and predicting chemotherapy benefit, but it is an expensive test and delays chemotherapy initiation. Magee equation 1 (with Ki67) and Magee equation 2 (without Ki67) are surrogate prediction models which incorporate standard histologic variables to estimate Oncotype DX recurrence score (RS). The objectives of this project are to independently examine if the RS calculated by Magee equation 1 or 2 is highly correlative to Oncotype DX RS and to identify the most accurate and cost-effective Magee equation for clinical use in Canadian population.

**Design:** We identified 253 cases of ER positive breast cancer with available Oncotype DX results from 2010 to 2018. All those cases have immunohistochemistry (IHC) stains for ER, PR and HER2. We will review all the cases and determine the Nottingham score, ER H-score, PR H-score, HER2 status, and tumor size. We will select the appropriate tumor area and perform IHC stains for Ki67. Automated computer based scoring will be used for Ki67. We will calculate RS with both Magee equation 1 and 2. Following the criteria set by Oncotype DX RS for low (<18), intermediate (18-30), and high (>30) risk categories, RS from two Magee equations will be assigned into those categories and compared with Oncotype results. The Pearson correlation coefficient between Oncotype DX and Magee equation 1 or 2 will be calculated. For the pilot study, we randomly selected 50 cases, reviewed their clinicopathological parameters, and calculated RS using the Magee equation 2. We compared the RS calculated by Magee equation 2 with the Oncotype DX RS.

**Results:** Results: For the 50 cases in the pilot study, following the criteria set by Oncotype DX RS for low, intermediate, and high risk categories, there is an overall 68% (34/50) agreement between Magee equation 2 RS and Oncotype DX RS (see Table). The correlation efficient is 0.98 ($p<0.0001$, see Figure).

| | Oncotype DX RS | | |
|---|---|---|---|
| Magee 2 RS | <18 | 18-30 | >30 |
| <18 | 24 | 5 | 0 |
| 18-30 | 7 | 8 | 2 |
| >30 | 1 | 1 | 2 |

Figure 1 - 124




**Conclusions:** In our pilot study, Magee equation 2 can predict Oncotype DX RS. We will continue our project to reach the final conclusion and find out if Magee equation 1 is superior to Magee equation 2 with automated computer based scoring of Ki67 adding to the equation.

## 125 Low pTEN Expression is Associated with Recurrence and Elevated Moesin Expression in Invasive Breast Carcinoma

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**Disclosures:** Lina Chen: None; Victoria Hoskin: None; Abdi Ghaffari: None; Sandip Sengupta: None; Yolanda Madarnas: None; Peter Greer: None; Bruce Elliott: None; Sonal Varma: None

**Background:** Loss of the tumor suppressor pTEN is linked with disease aggressiveness in many cancer types including breast cancer (BC). Recent studies also show an association of pTEN loss with triple-negative BC (TNBC), although its prognostic significance is not yet well established. The ERM (ezrin-radixin-moesin) family of cytoskeleton proteins are known to regulate PI3K/AKT signaling downstream of pTEN as well as metastasis in preclinical studies. Moesin in particular is also a marker of TN/basal-like BC (EGFR+, CK5+) and may be linked to pTEN loss. Recent sub-typing of basal-like BCs show significantly worse response to chemotherapy in the basal-like 2 (BL-2) subgroup which is characterized as TN, pTEN$^{low}$ and EGFR/CK5+.

**Design:** Tissue microarrays were constructed from FFPE tissues of 350 women with a primary diagnosis of invasive breast carcinoma between 2000 to 2008. Protein expression of pTEN, CK5, EGFR, ER, PR, HER2 and moesin were evaluated by immunohistochemistry. H-Scores were calculated for pTEN and Moesin using the Halo$^{TM}$ automated platform and median H-Score values were used as a cut-off for low versus high biomarker status. Recurrence-free survival (RFS) and overall survival (OS) were assessed using Kaplan-Meier survival analysis.

**Results:** Our data show that low pTEN expression was associated with lower RFS in all-comer (p=0.0096, Figure 1) and in TNBC cases (p=0.0281). No association between pTEN and OS in all-comer cases was observed (p=0.6947), however a trend towards significance was noted in TNBC cases (p=0.0813). Lower pTEN expression correlated with high moesin in all cases (p=0.0308) and with CK5/EGFR+ (basal) TNBC cases (p=0.0027, Figure 2). Furthermore, 60% (6/10) of CK5/EGFR+ TN cases were considered pTEN$^{low}$Moesin$^{high}$, compared to 6.3% (2/32) of CK5/EGFR- TN cases (p=0.0009).

Figure 1 - 125

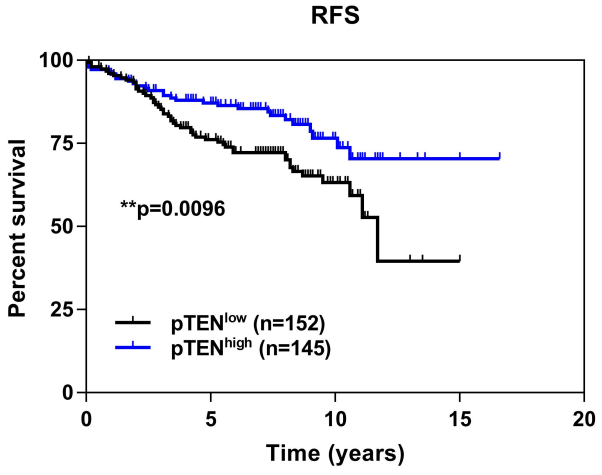


Figure 1 Low pTEN expression associated with lower RFS in all-comers

Figure 2 - 125

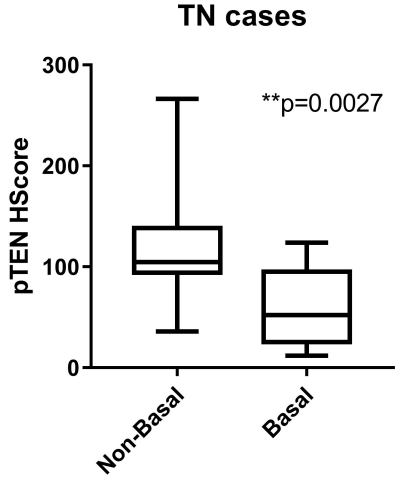


Figure 2 Low pTEN expression associated with basal-like TNBC cases

**Conclusions:** We have validated the observation that low pTEN protein expression is a prognostic indicator for RFS in patients with invasive breast carcinoma, particularly in TNBC. Our results also show an association between low pTEN and high Moesin levels in CK5/EGFR positive TNBC, likely representing a BL-2 subtype with worse prognosis and suggest that moesin may represent a novel therapeutic target for these cancers. (Funded by an OMPRN Stream 2 grant)

## 126 Rosai-Dorfman Disease of Breast. Clinicopathological Analysis of 6 Cases.

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**Disclosures:** Esther Cheng: None; AbdulAziz Al-Malki: None; Dean Joelson: None; Syed Hoda: None

**Background:** Rosai-Dorfman disease (RDD) is a rare disorder mainly characterized by massive lymphadenopathy due to histiocytic proliferation. The breast parenchyma is uncommonly involved. RDD of breast (RDD-B) has been reported as individual cases or as small series (largest: 7 cases, by eponymous authors, *Am J Surg Pathol*1997;21:664–668).

**Design:** Departmental (including consultation) database was searched for RDD-B from 1998 to 2018. Clinicopathological material was reviewed. Diagnostic criteria outlined in *Cancer* 1972;30:1174–1188 were used.

**Results:** Six (consultation) cases of RDD-B were identified. Mean age of patients was 42.5 (range 25-61). RDD-B was in left breast: 5, right breast: 1. 5 presented with a mass, 1 with imaging abnormality. 1 patient had, otherwise unexplained, fever. Mean mass size was 2.6 cm (range 0.8-5.0 cm). 1 patient had synchronous RDD of skin. Consulting pathologists' differential diagnosis included fat necrosis, mastitis, and inflammatory pseudotumour. RDD-B cases showed sheets of large polygonal histiocytes (Figure 1) that were S100 protein (+) (Figure 2). Emperipolesis and mitoses were rare. 1 case showed diffuse, and 1 focal, spindling of histiocytes. 1 patient had near-synchronous ipsilateral invasive carcinoma in another quadrant (diagnosed 11 days earlier). All RDD-B lesions were excised. Follow-up (mean 39 mo, range: 3-66 mo) showed neither residual/recurrent RDD-B nor RDD elsewhere (including in the case with skin involvement).

Figure 1 - 126

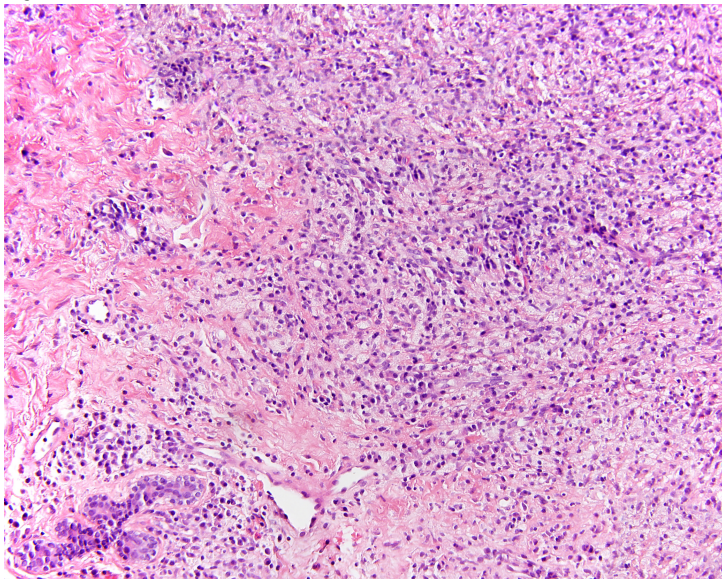
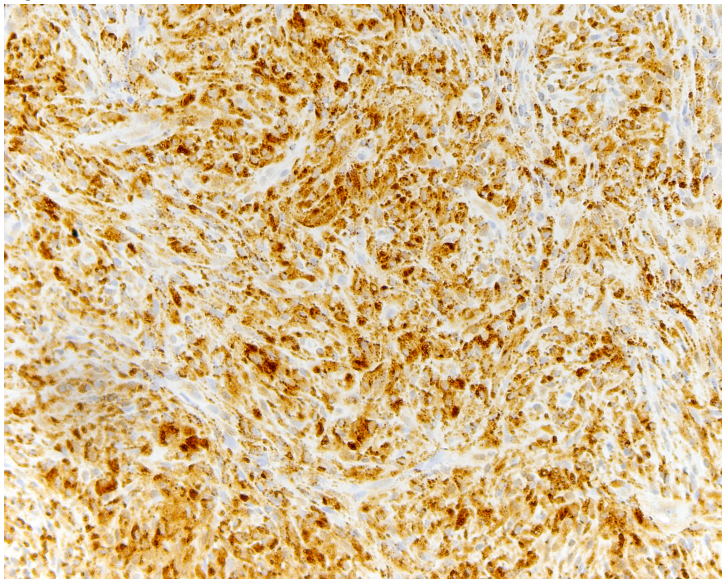


Figure 2 - 126



**Conclusions:** RDD-B (i) usually presents with a mass, (ii) may or may not be confined to breast, (iii) shows low-morbidity, (iv) can be conservatively managed, and (v) is self-limited. This study confirms earlier reports that excision of RDD-B appears to be curative.

## 127 Impact of 2018 CAP/ASCO guidelines for HER2 scoring in breast carcinomas- A single institution's experience with Dual in-situ hybridization (DISH) methodology as first line testing

Mamatha Chivukula[1], Andrea Metkus[2], Brown Jennifer[2], Kenneth Adler[2], Gautam Bulusu[3], Keith Duncan[4]
*[1]Mills-Peninsula Hospital, Sutter Health Affiliate, Cupertino, CA, [2]Dorothy Schneider Cancer Center, Mills-Peninsula Hospital, Sutter Health Affiliate, Burlingame, CA, [3]Mills-Peninsula Hospital, Sutter Health Affiliate, Burlingame, CA, [4]Mills-Peninsula Hospital, Sutter Health Affiliate, Hillsborough, CA*

**Disclosures:** Mamatha Chivukula: None; Keith Duncan: None

**Background:** The 2018 CAP/ASCO guidelines published recently has significantly modified the scoring of HER2 tests because of the introduction of "special groups". The expert panel in the new guidelines preferentially recommends the use of dual-probe instead of single probe ISH assays. As the "equivocal" category is eliminated in new guidelines, they suggest that "additional work up required" in selective

groups to more accurately determine HER2 status. The purpose of our study is to retrospectively evaluate the impact of the new HER2 guidelines on a subset of invasive breast carcinomas tested by DISH methodology as first line assay with emphasis on 'equivocal" category.

**Design:** 895 cases (2013-2017) were selected for retrospective review based on 2017 CAP/ASCO guidelines. Cases were re-classified based on the new 2018 guidelines. Clinico- pathological features were evaluated. HER2 “equivocal” cases were based on the new scoring system, Alternate reflex testing by FISH and/or IHC methodology were compared. Overall, disease free and survival rates were calculated.

**Results:** Non-amplified cases accounted for 86% of the cases (770/825); amplified cases represented 8.9% of the cases (80/825); and equivocal cases were 3.4% of the cases (28/825). Most of the cases were primary tumors 97.9% (808/825), while remaining 2.1% (17/825) were metastatic cancers. Alternate reflex HER2 testing by FISH and/or IHC was performed in 5% of the cases (46/895). In applying the new scoring system in the 2013-Equivocal group (n=28) and utilizing alternate FISH testing, 7.1% (2/28) cases were re-classified as HER2 positive; 43% (12/28) were classified as negative and 50% (14/28) required additional work up including repeat testing on alternate specimens.

**Conclusions:** Overall, 50% of equivocal cases were resolved with additional reflex testing; 7.1% of the cases (2/28) were re-classified as HER2 positive and therefore eligible for target-therapy. Alternate testing with FISH/IHC and additional work-up was of great benefit in a subset of cases in avoiding false negatives. However, caution needs to be taken to avoid over-use of these tests. The study is on-going. As we understand and implement the latest HER2 guidelines, additional prospective clinical trials are required to support our study findings.

## 128 Impact of Platinum-Based Neoadjuvant Chemotherapy in Histopathological Parameters in Triple Negative Breast Cancer

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**Disclosures:** Nikaoly Ciriaco: None; Adriana Zucchiatti Ll.: None; Armando Reques: None; Santiago Ramon Y Cajal: None; Esther Zamora: None; Vicente Peg: None

**Background:** Triple-negative breast cancers (TNBC) account for 10-20% of all breast tumors. Although TNBC are more aggressive than others breast cancer subtypes, they have higher sensitivity to neoadjuvant chemotherapy (NACT) and higher rates of pathologic complete response (pCR). The role of platinum agents (PA) in addition to standard NACT in TNBC has been investigated and studies have shown that its use was associated with significant increased of pCR rates but its impact on survival is still unclear. To our knowledge no studies have been done to evaluate its effect on the tumor and residual disease. The aim of our study is to compare histopathological parameters and Ki67 in a series of TNBC patients treated or not with PA in addition to standard NACT.

**Design:** This is a retrospective study of 107 TNBC patients diagnosed between 2004-2017 and treated with NACT in Vall d’Hebron University Hospital. Histological tumor type, tumor grade, tumor size, Ki67 rates (pre and post NACT), Residual Cancer Burden (RCB) and pCR were reviewed and statistical analysis was realized to evaluate the differences between patients treated with PA and those who were not.

**Results:** The majority of the tumors were invasive carcinomas NOS (88,7%) histological grade 3 (67,6%). BRCA mutations were evaluated in 25 of the patients (23,4%) harboring 7 (28%) patients with BRCA1 mutations and 1 (4%) with BRCA2 mutations. 28 (26,7%) of the patients received PA in addition to conventional NACT.

Reduction of Ki67 between pre- and post-NACT in breast cancer patients with non-pCR was higher after PA treatment (mean 53.3% vs 33%, p=0.019). The number of pCR was more elevated in the PA group (46.4%) compared to conventional NACT (27.3%) even if this finding didn’t reach a statistically significant p-value (0,063). No differences were observed in terms on histological grade, tumor size or RCB score after NACT between patients who received PA or not.

**Conclusions:** Our study shows that patients who received PA NACT have a higher reduction of Ki67 in residual disease, suggesting a beneficial impact in survival (as it has been demonstrated for Luminal tumors). Additional trials are still ongoing (ie. E1131, NCT02445391) to better understand the role of PA therapy.

## 129 Sox10 and p16 are Co-expressed by Immunohistochemistry in Triple Negative Breast Cancer (TNBC)

Kimberly Cole[1], Parker Wilson[2], Esther Yoon[1], Lina Irshaid[3], Marguerite Pinto[4], Malini Harigopal[1]
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**Disclosures:** Kimberly Cole: None; Parker Wilson: None; Esther Yoon: None; Lina Irshaid: None; Marguerite Pinto: None; Malini Harigopal: None

**Background:** Sox10 is a transcription factor important for survival and differentiation of neural crest cell lineages. The $p16^{INK4a}$ pathway is involved in cell-cycle regulation and plays a key role in tumorigenesis. Additionally, there is some evidence in melanoma cells lines that Sox10 interacts with the $p16^{INK4a}$ pathway, and therefore also plays a role in regulating cell cycle checkpoints, suggesting that these markers may be co-expressed in some tumors. In recent years, studies have shown that the immunohistochemical expression of both Sox10 and p16 is associated with triple negative breast cancer and that p16 overexpression predicts a poor outcome in all invasive breast cancers. Both Sox10 and p16 are potentially useful predictive and/or prognostic biomarkers and possible therapeutic targets. Here, we report on the co-expression of p16 and Sox10 in triple negative breast cancers.

**Design:** Tissue microarrays containing 277 primary triple negative invasive breast cancers and 643 primary invasive ductal carcinomas, NOS were stained for Sox10 and p16 immunohistochemistry. The extent of nuclear Sox10 staining was scored as follows: 0 (0%), 1 (1-25%), 2 (25-50%), 3 (50-75%) and 4 (75-100); > 1% staining was scored as positive. Nuclear and cytoplasmic staining of p16 in > 1% of cells was considered positive, and the pattern was scored on a scale from 0-3 based on the extent of immunopositive cells 0 (0%); 1 (< 25%); 2 (25-75%) and 3 (>75%). Statistical analysis using spearman correlation was performed to determine the relationship of IHC expression patterns between Sox10 and p16 in TNBCs and non-TNBCs and Fisher's exact test for the distribution of expression.

**Results:** Overall, 80% of triple negative cancers demonstrated Sox10 immunoreactivity compared with 44% of non-triple negative cancers, and 56% of TNBCs were positive for p16 staining, versus 16% of non-triple negative cancers. Sox10 immunoreactivity also correlated with p16 positivity in TNBC with 70% of TNBCs immunoreactive for both markers (56 of 80 cases with both values, p=0.022), while no correlation was observed between the two markers in the non-triple negative cohort; only 7% of non-TNBCs were positive for both Sox10 and p16 (23 of 348 cases with values for both markers, p=0.626).

Figure 1 - 129

| | Score | TNBC | Non-TNBC |
|---|---|---|---|
| Sox10 p<0.0001 | Positive | 117 (80%) | 202 (44%) |
| | Negative | 30 (20%) | 262 (56%) |
| | Total | 147 | 464 |
| p16 p<0.0001 | Positive | 91 (56%) | 77 (16%) |
| | Negative | 72 (44%) | 408 (84%) |
| | Total | 163 | 485 |

**Conclusions:** Sox10 and p16 are preferentially expressed in TNBC compared with non-TNBC. Sox10 and p16 co-expression is significantly correlated in TNBC (and not in non-TNBC), potentially reflecting interactions in these oncogenic pathways in TNBC.

## 130 Breast Conserving Surgery With and Without Cavity Shave Margins Have Different Re-excision Rates but Similar Overall Cost

Lorraine Colon Cartagena[1], Patricija Zot[2], Raghavendra Pillappa[3], Michael Idowu[2], Valentina Robila[4]
*[1]Virginia Commonwealth University, Richmond, VA, [2]Virginia Commonwealth University Health System, Richmond, VA, [3]VCUHS, Glen Allen, VA, [4]Virginia Commonwealth University Health System, Glen Allen, VA*

**Disclosures:** Lorraine Colon Cartagena: None; Patricija Zot: None; Raghavendra Pillappa: None; Michael Idowu: None; Valentina Robila: None

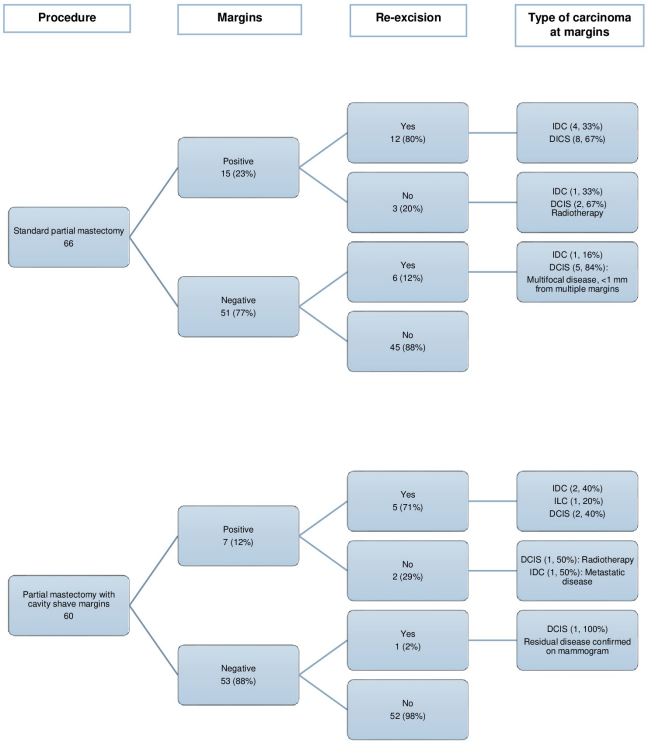
**Background:** While breast conserving surgery (BCS) provides a similar survival outcome as mastectomy, the margin status is a critical predictor of local recurrence. Variants of BCS include standard partial mastectomy (SPM), SPM with selective margins and SPM with circumferential cavity shave margins (CCS). Few studies acknowledge the effectiveness of these BCS variants with regards to rates of positive margins and re-excision. Therefore, we retrospectively evaluated our institutional outcomes to provide patients a higher level of care, decrease additional surgical interventions, complications and costs.

**Design:** 126 patients diagnosed with invasive ductal/ lobular carcinoma and/or ductal carcinoma in situ underwent BCS (2015-2018) at VCUHS, provided by six surgeons. A total of 66 patients had SPM or SPM with additional one to three margins, selected based on intraoperative radiological and gross evaluation. The remainder 60 patients underwent CCS. Data including pathological diagnosis, margin status on main specimen and additional margins, the rate and reason for re-excision, associated operation time and costs were recorded for both groups.

**Results:** Twice as many positive margins (PM) were reported in patients who underwent SPM (23%) compared with CCS (12%) (Fig. 1). In the former group, PM were predominantly noted on the main specimen, as the status of selective margins was negative in 35 (53%) patients. The re-excision rate for SPM and CCS was of 27% and 10%, respectively. Re-excision was performed almost exclusively for PM in the CCS group. However, 12% of SPM patients underwent re-excision of negative margins, for multifocal or moderate to extensive carcinoma less than 1 mm from the margins. For the initial procedure, CCS is on average 25% more costly than SPM (Table 1), mainly due to increased pathology costs. This is partially offset by costs associated with higher re-excision rate in SPM. However, in our cohort, there is no significant overall cost difference between the two arms.

| | **Standard partial mastectomy (with non-cavity shaved margins)** | **Standard partial mastectomy with cavity shave margins** |
|---|---|---|
| **Initial procedure** | | |
| Standard surgical cost | $2,621.00 | $2,621.00 |
| Pathology cost** | $2,216.00 ($1,705.00 - $2,728.00) | $3,751.00 |
| OR Time (hr:min) | 01:16 | 01:37 |
| **Initial Costs** | ~$4,837.00 x 66*= ~$319,242.00 | ~$6,372.00 x 60*= ~$382,320.00 |
| **Re-excision** | **Average additional margins: 2** | **Average additional margins: 1** |
| Standard surgical cost | $2,621.00 | $2,621.00 |
| Pathology cost | $682.00 ($341.00- $1,023.00) | $511.00 ($341.00 - $682.00) |
| OR Time (hr:min) | 01:25 | 00:54 |
| **Re-excision Costs** | ~$3,303.00 x 18* =~$59,454.00 | ~$3,132.00 x 6* = ~$18,792.00 |
| **Total Costs (initial cost + re-excision cost)** | ~$378,696.00 | ~$401,112.00 |

**Table 1. Cost comparison between SPM and CCS** *Indicates the number of patients undergoing a certain procedure. **Pathology cost charged as 88307 per specimen.




**Conclusions:** Our study shows that CCS reduces the rate of both positive margins and re-excision by approximately 50% when compared with SPM. Also, the rate of re-excision for negative but close margins is significantly reduced in this group. However, from a hospital perspective, the overall cost associated with either procedure is approximately similar.

## 131 Differences in Immunohistochemistry Utilization by General and Breast Subspecialty Pathologists at a Large Academic Institution

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**Disclosures:** Margaret Compton: None; Melissa Hogan: None; Emily Reisenbichler: None

**Background:** While hematoxylin and eosin (H&E) morphology is the gold standard for diagnostic breast pathology, immunohistochemistry (IHC) can be employed to aid in diagnostically challenging cases. The myoepithelial marker, p63, is one of the most commonly utilized markers in breast pathology, particularly helpful in distinguishing invasive carcinoma from ductal carcinoma in situ (DCIS) and benign mimic of invasion. It is thought that those less familiar with breast pathology may utilize IHC more frequently than those with subspecialty breast training. As many large academic institutions transition to subspecialty sign out, changes in IHC use may accompany this new sign out model. We sought to compare p63 use in breast biopsies by general and breast pathologists, with an emphasis on which specific types of lesions required IHC for diagnosis.

**Design:** The pathology database was searched over a 6-year period for cases reporting “p63” results in breast needle biopsy reports. Pathology reports were reviewed and the final diagnosis rendered was noted. Sign out by a subspecialty trained breast pathologist (BP) or by a general pathologist (GP) in each case was documented.

**Results:** 6671 total number of biopsies were reviewed (Table 1) and p63 performed on 130 (1.9%). Diagnoses rendered on stained cases were most frequently invasive carcinomas of no special type (n=61), followed by benign mimics of invasion such as sclerosing lesions or papillary lesions (n=40) and DCIS (n=22). P63 was infrequently used in the diagnosis of other malignant tumors such as metastases and metaplastic carcinomas (n=8)

P63 was ordered more frequently by BP (2.5% of cores) compared to GP who ordered the stain on 1.2% of cores (p<0.0001). The most frequent utilization of p63 by GP was on benign lesions (48%) followed by invasive carcinomas (33%) while BP most frequently ordered p63 on invasive carcinomas (52%) and DCIS (22%). Looking specifically at individual BP p63 utilization, there was no correlation with years

of practice experience by BP and the number of cases ordered. Those with the most and the least years of practice experience ordered p63 at similar frequencies.

| | General Pathologists | Breast Pathologists |
|---|---|---|
| **All cores reviewed** | 2844 | 3827 |
| **All p63 ordered** | 33 | 97 |
| **Final Diagnosis:** | | |
| **Invasive** | 11 (33%) | 50 (52%) |
| **DCIS** | 1 (3%) | 21 (22%) |
| **Other malignant** | 6 (18%) | 2 (2%) |
| **Benign** | 16 (48%) | 24 (25%) |

**Conclusions:** While IHC use may be thought to be most helpful to those with less experience or knowledge in breast pathology, these results suggest that utilization is increased with subspecialty training, particularly in the evaluation of invasive carcinomas.

## 132 LAG-3 Inhibitors in Breast Cancer: An Option for Patients Lagging Behind in Immunotherapy Response?

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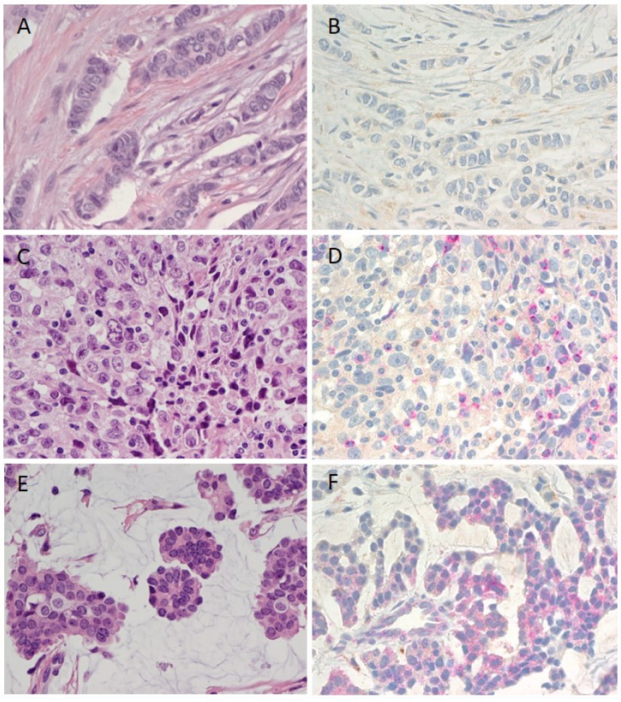
**Disclosures:** Michael Crawford: None; Anne Mills: None

**Background:** LAG-3 is a checkpoint molecule with similar immune inhibitory function to PD-1/PD-L1. Emerging immunotherapies targeting LAG-3 are in clinical trials for a variety of tumor types, including breast cancer. However, expression of LAG-3 in breast cancer has not been thoroughly investigated, and the relationship between LAG-3 expression in tumor cells and tumor-associated inflammation has not been correlated with response to anti-LAG-3 immunotherapies.

**Design:** LAG-3 immunohistochemistry was performed on two tissue microarrays containing 110 breast carcinomas (4 x 0.6 mm cores/case) including a variety of histologic subtypes. Immunohistochemical staining was scored separately in tumor-associated lymphocytes (TALs) and in tumor cells. LAG-3-positive TALs were manually quantitated, and tumor cells were semi-quantitatively scored based on percentage positivity (1-5%; 6-10%; 11-25%; 26-50%; >50%). Statistical analysis was performed using the Fischer's two-tailed Exact Test.

**Results:** LAG-3+ TALs were identified in 36% (40/110) of all breast cancers, with a mean per HPF of 11 (range: 0-180). The presence of LAG-3-positive TALs was significantly higher in triple-negative vs. hormone-receptor-positive cancers [58% (19/33) and 22% (16/71), $p$=0.0007] and in Grade 3 vs 1/2 [63% (27/43) vs. 19% (13/67), $p$=<0.0001]. Tumoral LAG-3 expression was present in 25% of cases (28/110), 21% (6/28) of which had >50% staining. Tumoral expression was non-significantly higher among triple-negative vs. hormone receptor-positive cancers [33% (11/33) vs. 21% (15/71), $p$=0.2252); however, expression rates were comparable among Grade 3 vs. 1/2 [26% (11/43) vs. 25% (17/67),$p$=1.0]. Tumoral expression was particularly high among medullary-like (64%, 7/11), mucinous (71%, 10/14), and apocrine (33%, 3/9) histologies; these tumor types comprised all cases with >50% expression. In contrast, only 10% (4/41) of conventional ductal and 14% (4/28) lobular cancers were LAG-3 positive in tumor cells.

Figure 1 – 132




**Patterns of LAG-3 Expression in Breast Cancer**: Most breast carcinomas were negative for LAG-3 in both TALs and in tumor cells, as with the ductal carcinoma pictured in A/B. LAG-3+ TALs were more common in high-grade, triple-negative carcinomas like the case pictured in C/D. Tumoral LAG-3 expression was seen in a subset of cancers across grades and hormone receptor statuses, including many mucinous cases like the one pictured in E/F. (A/C/E: H&E. B/D/F: LAG-3, pink stain. 400x).

**Conclusions:** LAG-3 expression is seen in both TALs and tumor cells in a subset of breast cancers. While LAG-3+ TALs are higher among high-grade and triple-negative cancers relative to low-grade and hormone receptor-positive cases, tumoral expression is identified across grades and hormone statuses. These data suggest a possible role for LAG-3 checkpoint inhibition in breast cancer patients, either alone or in combination with existing immunotherapies.

## 133 Recurrent MED12 Exon 2 Mutations in Complex Fibroadenomas of the Breast

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**Disclosures:** Edaise Da Silva: None; Ana Paula Martins Sebastiao: None; Melissa Murray: None; Catarina Silveira: None; Fresia Pareja: None; Britta Weigelt: None; Edi Brogi: None; Jorge Reis-Filho: *Advisory Board Member*, Volition Rx; *Advisory Board Member*, Paige.AI; *Consultant*, Goldman Sachs; Hong (Amy) Zhang: None

**Background:** Breast fibroepithelial lesions encompass a spectrum of entities including fibroadenomas (FAs) and phyllodes tumors (PTs). FAs and PTs harbor recurrent *MED12* exon 2 mutations, whereas *TERT* promoter hotspot mutations have been documented in PTs. FAs comprise multiple subtypes, including conventional FAs and histological variants, such as complex fibroadenomas (CFAs), defined by the presence of sclerosing adenosis, papillary apocrine metaplasia, cysts or epithelial calcifications. CFAs are associated with an increased risk for subsequent development of breast cancer. Whether CFAs would be related to conventional FAs and PTs remains to be elucidated. Here we sought to determine the frequency of *MED12* exon 2 mutations and *TERT* promoter hotspot mutations in CFAs.

**Design:** CFAs were retrieved from the pathology archives of the authors' institution, centrally reviewed by four pathologists for the diagnosis of CFA. The stromal component from representative sections of CFAs was microdissected. DNA extracted from the stromal component of all cases was subjected to Sanger sequencing analysis of exon 2 of *MED12* and the *TERT* promoter hotspot locus.

**Results:** Our study included 21 CFAs from patients with a median age of 45 years at diagnosis (range 33 to 82). *MED12* exon 2 mutations were identified in the stromal component of 24% (5/21) of CFAs. Four CFAs harbored missense mutations affecting codon 44

of *MED12* including three p.Gly44Ser and one p.Gly44Val mutations. One CFA had a *MED12* p.Leu36Arg mutation, predicted to be pathogenic. No *TERT* promoter hotspot mutations were detected in the CFAs analyzed.

**Conclusions:** We have identified recurrent *MED12* exon 2 mutations in the stromal component of CFAs. Akin to other histologic variants of FAs, the frequency of *MED12* exon 2 mutations we observed in CFAs was lower than that reported in conventional FAs. These findings suggest that at least a subset of CFAs may be genetically similar to conventional FAs. The genetic underpinning of CFAs lacking *MED12* exon 2 mutations warrants further investigation.

## 134 Correlation of Asparagine Synthetase with Histological Grade of Breast Cancer: Increased Evidence for Asparagine Deprivation Therapies?

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**Disclosures:** Leslie Dalton: None

**Background:** Asparagine synthetase (ASNS) was identified as being an important component of a protein-based profile to predict histologic grade. Literature is sparse on the role of ASNS in breast cancer. Based on studies in mice, recent attention has been directed toward ASNS in regard to possible use of asparagine depletion therapies as a specific treatment for breast cancer.

**Design:** 769 invasive breast cancer cases from the TCGA provisional dataset (cBioPortal.org) had both reverse phase protein assay (RPPA) data (214 proteins) and evaluable whole slides images (WSI). From WSI, a grade was assigned to each case, and grade then served as a target variable with proteins as predictors. Feature selection used an information theory (IT) approach, and significance of those features selected was determined by ridge regression (RR).

**Results:** The IT algorithm selected Cyclin_B1 (CB), ER_alpha (ER), and ASNS as most informative. By RR, all were highly significant (each $p < 0.00001$) in building a multivariate model to predict cancer grade. Yet, it was ASNS which garnered attention, and not so much the model. Spearman correlation of ASNS-RPPA with CB showed rho =0.56 ($p<0.00001$) and ASNS-RPPA with ER corresponded to rho= -0.34 ($p<0.00001$). At level of quantile discretization, ASNS was highly correlated with grade (rho= 0.49, $p<0.00001$). Tumors with highest quantile expression of ASNS-RPPA were 79% high grade. ASNS-RPPA expression was also highly correlated with ASNS-mRNA (rho=0.76, $p<0.00001$). ASNS-mRNA respectively had rho= -0.51, 0.43 (both $p<0.00001$) with mRNA of ESR1 and Ki-67. Ki-67 expression by RPPA was not available in the TCGA data.

**Conclusions:** The TCGA dataset was troubled by survival data being limited to short term follow-up and having excessive censoring. However, grade has been a significant predictor of patient outcome in innumerable studies, and has been used as a target variable to define competent molecular profiles. Findings here show a significant linkage of ASNS with grade, and therefore a high probability of an ability for ASNS to also predict patient outcome. ASNS might be regarded as just another proliferation marker, but level of ASNS expression might be indicative of a need for, and success of, asparagine depletion therapies. Such therapies may be as simple as a change in diet.

## 135 Histopathologic Features of Breast Tumors in Patients with BRCA2 High Penetrance Germ Line Mutations Suggest a Morphologic Heterogeneity

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**Disclosures:** Alexander Damron: None; Katherine Nathanson: None; Kara Maxwell: None; Payal Shah: None; Susan Domchek: None; Ira Bleiweiss: None; Anupma Nayak: None

**Background:** Hereditary breast cancer comprises an estimated 5-12% of all breast cancers that arise in individuals, of which a significant proportion are attributed to mutations in the *BRCA1* and *BRCA2* genes. There have been several studies elucidating the unique molecular profile of *BRCA1* positive cancers, as well as the unique immunophenotypic and histologic findings associated with *BRCA1* germline mutations; however, the histopathologic features of breast cancer associated with a germline *BRCA2* mutation is less clear. In fact, *BRCA2* positive breast cancers are known to be molecularly heterogeneous compared to *BRCA1* germline mutations. Germline *BRCA2* mutations have been described as being associated with lobular cancers and extensive intraductal cancers; however, our study indicates a morphologic heterogeneity that may correlate with the molecular heterogeneity of these tumors.

**Design:** Seventy cases (71 distinct tumors, 3 male and 67 female) of breast cancer arising in known *BRCA2* germline mutation carriers were examined in this retrospective study. A review of the corresponding H&E-stained slides was performed for each case to identify histopathologic features associated with each tumor. Biomarker status was determined via review of original pathology reports of the corresponding tumors.

**Results:** The average patient age of the cases was 46 years. The tumors had a propensity to be high grade (poorly differentiated). Sixty-two tumors had complete biomarker data available; the majority of tumors were estrogen receptor positive (76.1%) and a minority of tumors were triple negative (12.7%). Three cases had bilateral disease (4.3%). The histologic features and receptor status of *BRCA2* associated tumors are summarized in Table 1.

| **Table 1 - Clinical and Histopathologic Features of BRCA-2 associated carcinoma** | |
|---|---|
| **N = 71 tumors (70 patients)** | |
| Age, median | 46 (range 25 to 84) |
| Male | 3 |
| Female | 67 |
| Average Tumor Size | 1.8cm (range 0.1 to 5.5cm) |
| **Histotype** | |
| IDC, NOS | 40 (56.3%) |
| IDC with mucinous features | 2 (2.8%) |
| IDC with micropapillary features | 9 (12.7%) |
| IDC with medullary features | 1 (1.4%) |
| IDC with anaplastic features | 2 (2.8%) |
| IDC with metaplastic features | 2 (2.8%) |
| IDC with lobular features | 4 (5.6%) |
| Mixed IDC and ILC | 3 (4.2%) |
| ILC, Classic type | 2 (2.8%) |
| ILC, pleomorphic type | 1 (1.4%) |
| Tubular Carcinoma | 1 (1.4%) |
| Tubulolobular carcinoma | 2 (2.8%) |
| Metastatic carcinoma to ovary | 1 (1.4%) |
| No residual invasive component | 1 (1.4%) |
| **Tumor Differentiation** | |
| Poorly differentiated (SBR 8-9) | 39 (54.9%) |
| Moderately Differentiated (SBR 6-7) | 27 (38.0%) |
| Well Differentiated (SBR 3-5) | 5 (7.1%) |
| **Biomarker Status** | |
| ER+PR±HER2- | 46 (64.8%) |
| ER+PR±HER2+ | 4 (5.6%) |
| ER-PR+HER2- | 1 (1.4%) |
| ER-PR±HER2+ | 2 (2.8%) |
| ER-PR-HER2- | 9 (12.7%) |
| Unknown/Incomplete | 9 (12.7%) |
| **Additional Findings** | |
| TILs >50% | 11 (15.5%) |
| Multifocality | 13 (18.3%) |
| Lymphatic invasion | 29 (40.8%) |
| Positive Axillary Lymph Nodes | 26 (36.6%) |
| Negative Axillary Lymph Nodes | 30 (42.3%) |
| Unknown Lymph Node Status | 15 (21.1%) |

**Conclusions:** Overall, *BRCA2* associated breast cancers show more variable histopathologic characteristics than previously described. Lobular carcinoma did not have a higher frequency than what is seen in sporadic cancer. In fact, ductal carcinomas of high grade were the predominant histologic type; however these tumors had a variety of additional histologic features among them. Unlike *BRCA1* associated tumors, tumor infiltrating lymphocytes (TILs) were not a frequent feature and biomarker status showed a propensity for ER+ tumors. Our findings suggest a histopathologic heterogeneity in *BRCA2* breast carcinomas, which may correlate with the molecular heterogeneity previously described.

## 136 Mammaprint: Histo-Pathologic Associations and Comparison to Magee Equations

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**Disclosures:** Stephanie David: None; Michelle Heayn: None; David Dabbs: None; Beth Clark: None; Rohit Bhargava: None

**Background:** Magee Equations (MEs) are multi-variable models that can estimate oncotype DX® (ODX) recurrence score and ME3 score of ≥31 has been shown to have independent chemo-predictive value (Farrugia DJ et al. Mod Pathol. 2017. PMID: 28548119). Mammaprint (MP) is an FDA cleared multi-gene assay indicated for prognostic use in early stage breast cancer, regardless of ER status. However, both MP and ODX are often used for making chemotherapy decisions (predictive use) in ER+ breast cancers in routine practice. There is considerable difference in risk assignment between ODX and MP (Dabbs DJ et al. J Clin Oncol. 2014(May 20);32,no.15_suppl:550), likely because risk of recurrence associated with ODX scores is after administration of 5 years of endocrine therapy and risk of recurrence associated with MP results is without any therapy. We have previously shown how MEs can be effectively used in routine practice in lieu of ODX (Bhargava R et al. USCAP 2018, abstract 148).

**Design:** The current study analyzed the clinical requests for MP testing at our institution from the last 2.5 years. A total of 160 cases (all ER+) were sent for MP testing. Thirteen cases were treated with neoadjuvant therapy, 8 with neoadjuvant chemotherapy (NACT) and 5 with neoadjuvant hormonal therapy (NAHT). All Magee equations were calculated on 147 non-neoadjuvant cases but only ME3 was calculated on 13 neoadjuvant cases. The associations between MP results with average ME scores and other variables are described. Responses to NACT on 8 cases were compared between MP results and ME3 scores.

**Results:** Please see table 1 for results for association of variables with Mammaprint results. All eight cases treated with neoadjuvant chemotherapy showed high MP result, but none achieved pCR, only one showed residual cancer burden (RCB)-1, 5 RCB-II, and 2 RCB-III. In contrast, only one case showed ME3 score >31 and this case showed RCB-1. The other 7 cases with RCB-II and RCB-III showed ME3 scores ranging from 17.9 to 30.3 with mean and median ME3 scores of 23.5 and 22 respectively.

Table 1: Associations between MP results and pathological variables

| Variables | MP high (n=75) | MP low (n=85) | p-value |
|---|---|---|---|
| Age (years) | 55.3 | 56.8 | 0.4605 |
| Nottingham Score[a] | 22 | 63 | <0.0001* |
| 4-6 | 45 | 22 | |
| 7-9 | | | |
| Nottingham Grade[a] | 2 | 14 | Ref |
| I | 35 | 62 | 0.0848 |
| II | 30 | 9 | <0.0001* |
| III | | | |
| ER H-score | 238 | 239 | 0.9550 |
| PR H-score | 134 | 168 | 0.0349* |
| HER2 status | 10 | 7 | 1.0000 (neg/eq vs pos) |
| Negative | 63 | 76 | |
| Equivocal | 2 | 2 | |
| Positive | | | |
| Ki-67 LI (%) | 32 | 18 | <0.0001* |
| Tumor size | 2.4 | 2.9 | 0.1116 |
| LN status | 1 | 4 | 0.7343 (neg vs pos) |
| ITC | 48 | 55 | |
| Negative | 25 | 26 | |
| Positive | 1 | 0 | |
| Unknown | | | |
| Tumor type | 64 | 63 | 0.1644 (ductal vs lob) |
| Ductal | 7 | 15 | |
| Lobular | 4 | 7 | |
| Mixed | | | |
| Average ME score | 15 | 34 | Ref |
| Less than 18 | 38 | 45 | 0.1001 |
| 18 to 25 | 15 | 6 | 0.0032* |
| >25 to <31 | 7 | 0 | 0.0007* |
| 31 or higher | | | |

[a]Nottingham score/grade excludes neoadjuvant chemotherapy cases. *Statistically significant. MP: Mammaprint; ME: Magee Equations.

**Conclusions:** High MP results are associated with high Nottingham grade, low PR H-score and high Ki-67 index. Increasing ME scores predicted for high MP results. Results on 8 cases subjected to NACT shows that MP-high doesn't always predict for chemotherapy benefit. In contrast, ME3 scores are highly chemo-predictive. MP is FDA cleared for prognostic use only and should not be used for chemo-prediction (especially in the neoadjuvant setting) in routine practice.

## 137 HER2 Status in Breast Carcinoma by Dual ISH in HER2 Equivocal IHC Population: Comparison Between 2013 and 2018 Guidelines in Reference Center

Paola De La Iglesia Niveyro[1], Alejandra Wernicke[2], Julieta Pandolfi[1], Ana Pastorutti[1], Hernan Garcia- Rivello[3]
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**Disclosures:** Paola De La Iglesia Niveyro: None; Julieta Pandolfi: None; Ana Pastorutti: None; Hernan Garcia- Rivello: None

**Background:** Her2 status is an important predictive marker in breast cancer. In our country, first tool for Her2 assessment is immunohistochemistry (IHC) and all cases with equivocal IHC results are referred for in situ hybridization (ISH). Recently released Her2 guidelines modify ISH interpretation with elimination of the equivocal ISH category and downgrade from positive to negative result samples with Her2/Cep17 ? 2 but with mean copy number < 4 copies/cell.

**Design:** We retrospectively investigated ISH results of 242 ish assays performed in our hospital between 2016 and 2017 with previous IHC equivocal (2+) result using a dual ish strategy (Pathvysion, Abbott). Between 50 and 100 cells were scored. All cases with Her2/cep17 between 1.8 and 2.2 were evaluated by 2 observers independently. Her2/Cep17 ratio, mean Her2 copy number, final result according to 2013 and 2018 guidelines and tumoral heterogeneity was assessed. Tumoral heterogeneity was defined as proportion of tumor cells with individual Her2/cep17 ? 2 within a sample. Cases were grouped in the following categories: Group 1, Her2/cep17 ratio ? 2 mean Her2 copies ? 4; Group 2 Her2/cep17 ratio ? 2 mean Her2 copies < 4, Group 3 Her2/cep17 ratio < 2 mean Her2 copies ? 6; Group 4 Her2/cep17 ratio < 2 mean Her2 copies ? 4 and < 6; Group 5 Her2/cep17 ratio < 2 mean Her2 copies < 4.

**Results:** Using 2013 guideline criteria ISH results were as following: NEGATIVE 82.2% (n=199), EQUIVOCAL 2.9% (n=7), POSITIVE 14.9% (n=36), according to 2018 guideline results were: NEGATIVE 88.4% (n=214), POSITIVE 11.6% (n=28). Differences were statistically significant between positive and negative results (chi square p<0.005). Distribution in groups is shown in table 1. Change from positive to negative result was observed in 8 cases (3.3%) and change from equivocal to negative in 7 cases (2.9%). Also, groups 2-4 were markedly heterogeneous (mean heterogeneity 0.38 and 0.14 respectively). Overall 15 out of 242 (6.2%) samples had a change in Her2 status.

Table 1

| GROUPS (2018 guideline) | n | % |
|---|---|---|
| Group 1 | 25 | 10.3 |
| Group 2 | 8 | 3.3 |
| Group 3 | 1 | 0.4 |
| Group 4 | 7 | 2.9 |
| Group 5 | 201 | 83.1 |
| Total | 242 | 100 |

**Conclusions:** IHC equivocal cases rely exclusively on ISH results to define Her2 status. Application of 2018 guideline criteria resulted in subtle but significant change in Her2 status (6%) with a decrease in positive cases and correspond to 2013 equivocal category and to samples in group 2 (Her2/cep17 ratio ? 2 mean Her2 copies < 4). ). Elimination of ISH equivocal category simplifies algorithm and avoids delay in definition of Her2 final status.

## 138 Association Between Obesity And Macrophagic Crown-Like Structures In Benign Breast Disease And Breast Neoplasia In African American Women

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**Disclosures:** Hany Deirawan: None; Michele Cote: None; Asra Shaik: None; Julie Ruterbusch: None; Ibrahim Tsolakian: None; Haidy El Azzamy: None; Jack MacLean: None; Sudeshna Bandyopadhyay: None; Rouba Ali-Fehmi: None

**Background:** African American women (AAW) have a high prevalence of obesity, an established risk factor for Breast Cancer (BrCa), they also have a higher mortality rate and worse BrCa outcomes. Recent reports suggest that obesity plays a role in the induction of a pro-inflammatory immune response. Mammary adipose tissue inflammation, depicted as macrophages encircling damaged adipocytes and

called crown-like structures (CLS-B), has been linked to obesity and hormonal abnormalities. The clinical significance of CLS-B in normal, premalignant and malignant tissue is not well characterized especially in AAW.

**Design:** Three age-matched groups [40 AAW with no history of BBD or BrCa from the Komen Normal Tissue Bank (KTB), 44 AAW with BBD who did not develop breast cancer (BBD control) and 45 AAW with BBD from the Detroit cohort who developed cancer (BBD case)] were identified. Disease-free areas of breast tissue were examined with H&E and immunohistochemically stained for CD68 for CLS-B. Association between breast disease status and CLS-B presence was assessed using chi-square test. Mantel-Haenszel test was used to adjust for BMI.

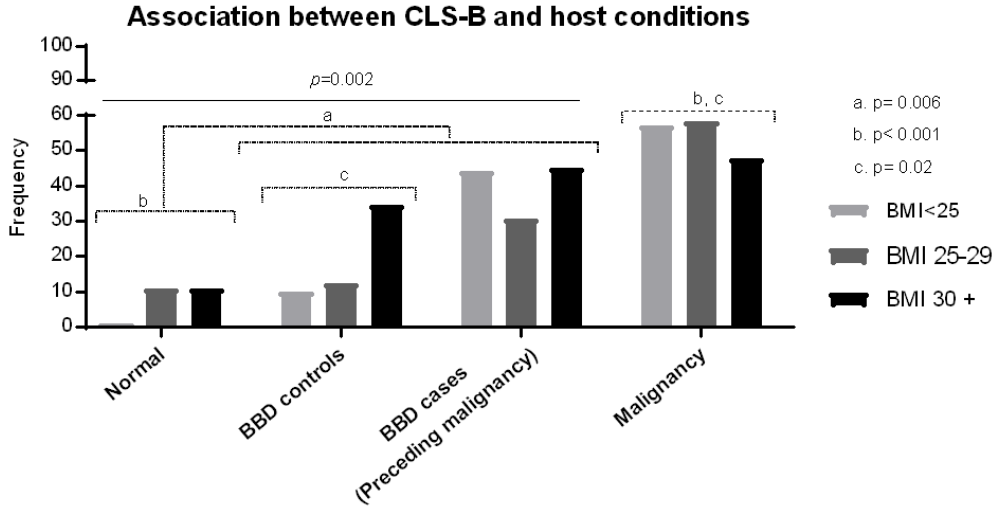
**Results:** The age-matched groups consisted of approximately <45: 15%, 45-55: 43%, and 55+: 42% year old AAW. Our study of KTB, BBD control and BBD case groups predominately consisted of overweight and obese women (see table 1)

CLS-B are infrequently identified (4/44=9.1%) in histologically normal breasts (KTB). In comparison, these lesions are significantly associated with BBD, regardless if the patient develops cancer or not (28/86=32.6%, p=0.006). In BBD, fewer BBD controls (11/45=24.4%) were CLS-B positive than BBD cases (18/44=40.9%) when adjusted for BMI (p=0.002, see figure). In addition, CLS-B lesions are significantly associated with malignant Breast biopsies (20/42=47.6%, p<0.001) compared to KTB and compared to BBD control (p= 0.02).

Finally, there was no significant difference in CLS between BBD cases and cancer cases (p=0.5)

| | Komen Normal Tissue Bank (KTB) | BBD controls | BBD cases | Cancer cases |
|---|---|---|---|---|
| N (%) with CLS-B presence | N=44 | N=44 | N=42 | N=42 |
| BMI<25 | 0/8 (0%) | 1/10 (10.0%) | 4/9 (44.4%) | 4/7 (57%) |
| BMI 25-29 | 1/9 (11.1%) | 1/8 (12.5%) | 4/13 (30.8%) | 5/12 (58.3%) |
| BMI 30+ | 3/27 (11.1%) | 9/26 (34.6%) | 9/20 (45.0%) | 11/23 (47.8%) |

Figure 1 - 138




**Conclusions:** This study confirms that macrophagic CLS-B are seen more frequently in association with obesity, benign and malignant breast disease. This increase in CLS-B incidence, more pronounced in BBD, was sustained overtime; It often preceded and accompanied malignancy. Macrophages are known surrogate for breast tumorigenesis and impaired immune microenvironment. CLS-B may serve as useful histological marker to identify AAW with disproportionately increased risk for breast cancer.

## 139 What COMETh after a positive margin on lumpectomy in patients with low risk DCIS (a COMET inclusion criteria)?

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**Disclosures:** Rosemarie Di Donato: None; Twisha Oza: None; Nebras Zeizafoun: None; Shabnam Jaffer: None

**Background:** One of the inclusion criteria of The Comparison of Operative to Monitoring and Endocrine Therapy (COMET) trial is patients with low risk DCIS who have a positive (+) margin on lumpectomy (lx). These patients are prospectively randomized to receive active surveillance or conventional treatment (lx, to achieve negative margins). We wanted to identify factors predictive of residual DCIS in this setting to reduce recurrence risk (RR).

**Design:** Using the pathology database, we retrospectively identified all lx performed for DCIS (426) that had either a + (29, 7%) *or close margin (within 1mm) (78, 18%)* that underwent subsequent re excision (reex) (n=107, 25%) from 2015 to date. All slides of the lx and reex were reviewed to confirm the diagnosis of DCIS and correlated with grade, pattern, size on lx, +margin extent and imaging.

**Results:** The incidence of + and close DCIS margins on lx is directly related to increasing grade (see table). The presence of residual DCIS post lx is directly related to margin involvement (+>close), increasing DCIS grade, and size (for low and intermediate grade (int) DCIS) which is usually underestimated on imaging. All low and most int DCIS with +margins but no residual DCIS on reex were diagnosed as atypical duct hyperplasia bordering to (ADH/) DCIS on core biopsy (cbx). Necrosis was absent in all low grade DCIS, present in about half of int (comedo=2cases) and almost all high grade (HG) DCIS, half comedo. All int DCIS cases with +margins and residual DCIS had necrosis and >1+/close margin. All cases of low and int DCIS were ER+, and 75% of high grade (HG). Excluding the HG DCIS cases, 14 (low=4, int=10) patients met criteria for the COMET trial of which 9(63%) had residual DCIS on reex (see table, underlined).

| GRADE (n=107/426 =25%) | +margin n=29 (27%) | residual DCIS s/p lx n=20 | ave size of DCIS on lx (residual DCIS vs nil) | 1mm margin n=78 (73%) | residual DCIS s/p lx n=40 | ave size of DCIS on lx (residual DCIS vs nil) |
|---|---|---|---|---|---|---|
| low 12/55 (22%) | 4 | 2 (50%) | 56mm>5mm | 8 | 4 (50%) | 26mm>14mm |
| int 49/224 (22%) | 10 | 7 (70%) | 36mm>8mm | 39 | 20 (52%) | 25mm>16mm |
| high 46/147 (31%) | 15 | 11 (73%) | 26mm=26mm | 31 | 16 (52%) | 24mm>22mm |

**Conclusions:** No upgrade to invasive carcinoma was identified. There is a 50% chance of residual DCIS on reex s/p lx for a + or close DCIS margin, which increases to 70% with +margins in int and HGDCIS. Factors predictive of residual DCIS post lx for +margins, include DCIS size (for low and int, >35mm), necrosis (int) and >1+/close margins (for int and HGDCIS), while presence of ADH/DCIS on cbx negatively correlated. These factors maybe useful to consider when selecting patients for the COMET trial with +lx DCIS margins, to prevent RR since 2/3rds of patients that met COMET criteria had residual DCIS in our series.

## 140 Retrospective Analysis of 158 Breast Cancer Cases of Group 4 HER2 FISH

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**Disclosures:** Qingqing Ding: None; Hongxia Sun: None; Bora Lim: None; James Crespo: None; Guilin Tang: None; Melissa Robinson: None; Aysegul Sahin: None; Hui Chen: None

**Background:** With the 2013 ASCO/CAP guideline for HER2 Testing in Breast Cancer, if HER2 ISH testing is ultimately deemed to be equivocal (*HER2*/CEP17 ratio <2.0 with *HER2* >=4.0 and < 6.0 signals/cell), even after reflex testing with an alternative assay, use of HER2-targeted therapy is an individualized decision based on patients' and oncologists' preferences. In the updated 2018 ASCO/CAP guidelines, the equivocal HER2 ISH, classified as group 4, combined with HER2 immunohistochemical results will be used to report a final positive or negative HER2 to facilitate the clinical decision. This study aims to retrospectively analyze breast cancer with equivocal HER2 ISH, to compare the ISH results in paired biopsy and resection samples, and to assess response to neoadjuvant treatment with or without anti-HER2 therapy.

**Design:** We collected 158 breast cancer patients with equivocal HER2 FISH result and 1+ or 2+ immunohistochemical stain on breast (n=104) and axillary lymph node (n=54) biopsy between 2014-2017. We recorded clinicopathologic features including age, clinical stage, hormone receptor status, tumor histologic grade, tumor Ki-67 proliferation rate, neoadjuvant therapy, chemo-agent received and pathological response. Patient age ranged from 30-91 years old (median 57 years), 155 females and 3 males.

**Results:** In this cohort, invasive carcinomas were predominantly ductal (except 5 lobular, 3 mucinous and 3 micropapillary) and Nottingham histologic grade 2 or 3 (only 5 grade 1). HER2 ISH was repeated in 58 patients in the resection specimen, 29 cases (50%) remained as group 4, 22 (38%) became negative (ratio <2 and *HER2* copy number<4), and 7 (12%) became positive (ratio >2 and *HER2* copy number>4). Among 73 patients received neoadjuvant therapy, 4 of 14 patients (29%) treated with additional anti-HER2 therapy got complete pathologic response (with no residual tumor in clinical and pathologic exams), in contrast 5 out of 59 patients (8%) without anti-HER2 therapy achieved complete clinical /pathologic response ($p<0.05$).

**Conclusions:** A small portion of group 4 cases were reclassified as positive HER2 ISH in the resection specimen. Increased chance of clinical/pathologic response after neoadjuvant therapy is observed in some group 4 cases with additional HER2–targeted therapy, but other factors such as tumor nuclear grade, ER status may contribute to this. Further evaluation and analysis will be followed.

## 141 Management of the Axilla Post Neoadjuvant Chemotherapy in Patients with Node Positive Breast Cancer: the Predictive Value of Sentinel Node Biopsy in Combination with Radiological Findings

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**Disclosures:** Kate Dinneen: None; Gibbons David: None

**Background:** Patients with a positive axillary lymph node (LN) who receive neoadjuvant chemotherapy (NACT) with a good radiological response may undergo sentinel lymph node biopsy (SLNB) to determine the need for axillary lymph node clearance (AXCL). This study investigates the predictive value of post NACT SLNB in patients known to be LN positive pre NACT, related to radiological axillary findings and primary tumour biomarker profile.

**Design:** The study group comprised 94 patients with positive pre NACT LN, diagnosed using ultrasound (US) guided fine needle aspiration cytology, treated at SVUH between June 2015 and July 2017. The axilla was examined pre and post NACT using magnetic resonance imaging (MRI) and US. Post NACT imaging findings directed initial surgical management of the axilla and were correlated with LN status. Tumour biomarker profile was 46 hormone receptor (HR)+ HER2-, 38 HER2+ and 12 triple negative (TN). Two patients had multifocal disease.

**Results:** Following post NACT imaging 50/94 patients (53%) had SLNB: 30 patients (60%) had positive SLNB and 26 progressed to AXCL with further nodal disease identified in 9 (35%). 34/44 patients (77%) who underwent direct AXCL had residual nodal disease. Post NACT LN positivity was associated with HR+HER2- biomarker profile ($p=0.013$) (Table 1). Improvement in MRI and US appearances were not significantly associated with SLNB negativity ($p=0.956$).

| | HR+HER2- | HER2+ | TN | Total |
|---|---|---|---|---|
| LN+ | 38 | 20 | 8 | 66 |
| LN- | 8 | 18 | 4 | 30 |

Table 1: Correlation between tumour biomarker profile and LN status post NACT. Two patients had two tumours with different biomarker profiles. *LN = lymph node; HR = hormone receptor; TN = triple negative.*

**Conclusions:** Our findings indicate that there is a high incidence of post NACT SLNB positivity in patients with positive pre NACT LN, regardless of imaging findings. There is a significant association between HR+HER2- biomarker profile and LN positivity, compared with HER2+ and TN tumours. Our data suggests that patients with a positive LN pre-NACT should have pathological assessment of the axilla post-NACT.

## 142 GLUT1 Pathway Hyperactivity and Cell Cycle Arrest in Micropapillary Breast Carcinoma (IMPC)

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

**Background:** IMPC is a special subtype of carcinoma associated with increased LVI, nodal metastases, and poor prognosis. The micropapillary components (MP) are solid or hollow clusters with inverted cell polarity and loss of stromal attachment. In the absence of stromal contact or a fibrovascular core, it is unclear how the IMPC survive, grow, and spread. Altered glucose metabolism is observed in many different tumor types, but has not been previously studied in IMPC. In this study, we investigated the role of the facilitative glucose transporter 1 (GLUT1) in IMPC.

**Design:** We assessed GLUT1 expression by IHC in 154 breast carcinomas using tissue microarrays (TMA) enriched for IMPC. GLUT1 expression level was determined by H-score method as low, intermediate, or strong expression in IMPC and invasive ductal carcinoma (IDC). Ki-67 proliferative index (PI) was assessed in GLUT1-expressing components. We assessed GLUT1, EMA, and E-cadherin expression in resection specimens from 6 cases of pure IMPC and 2 cases of IDC with micropapillary features (IDC-MP). GLUT1 expression was also assessed in breast cancer cell lines UACC-812, MDA-MB-453, and the reported MCF-7-spheroid IMPC-like model.

**Results:** All IMPC cases showed either low, intermediate, or strong membrane GLUT1 expression (luminal-facing and intercellular; 21/21, 100%). Only a subset of IDC showed membrane GLUT1 expression (33/133, 24.8%). The Ki-67 PI of IDC was higher than IMPC (33.2% vs. 8.9%, p<.005). Whereas GLUT1 expression-level positively correlated with proliferation in IDC, it inversely correlated with proliferation in IPMC (Ki-67 PI: GLUT1-strong IDC=58.6% vs. GLUT1-low IDC=17.3%, p<.005; GLUT1-low IMPC=9.6% vs. GLUT1-strong IPMC=4.3%, p<.005). GLUT1 expression in IMPC was seen in all IMPC/IDC-MP resection specimens and correlated with inverted-EMA/strong-E-cadherin staining. In IDC-MP, the Ki-67 PI of GLUT1+ MP was lower than areas of adjacent IDC (2.96% vs. 12.8%, p<0.05). Strong GLUT1 expression was observed in MCF7-spheroid IMPC-like cells, whereas no/faint-cytoplasmic expression was detected in UACC-812 and MDA-MB-453 cells.

**Conclusions:** Our current study shows GLUT1 upregulation in all IMPC/IDC-MP cases and an *in vitro* IMPC-like model, indicating altered glucose metabolism in these tumors. Whereas GLUT1 correlates with increased proliferation of IDC, higher levels in IMPC are associated with an arrested cell-state. This may explain the ability of IMPC to generate metastases, evade PET scans, and survive anti-cancer therapies.

## 143 The Immune Checkpoint TIM-3: A Therapeutic Target for Low-Grade and Hormone Receptor-Positive Breast Cancers

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**Disclosures:** Anna Dusenbery: None; Anne Mills: None

**Background:** Immunotherapy targeting the PD-1/PD-L1 immune checkpoint axis has shown promise in a subset of breast carcinomas, particularly among high-grade, hormone receptor-negative cancers. However, the majority of breast cancers show no durable benefit to this therapeutic approach, and its role in low-grade, hormone receptor-positive breast carcinomas has been particularly limited. TIM-3 represents another immune inhibitory checkpoint molecule with expression demonstrated in a variety of tumor types, and several drugs targeting TIM-3 are being investigated in clinical trials. However, little is known about TIM-3 expression in breast carcinomas.

**Design:** TIM-3 immunohistochemistry was performed on two tissue microarrays containing 123 breast carcinomas (4 x 0.6 mm cores/case) including a variety of histologic subtypes. Immunohistochemical staining was scored based on the percentage of tumor cells demonstrating cytoplasmic expression. Statistical analysis was performed using the Fischer's Two-Tailed Exact Test.

**Results:** The majority of breast cancers (111/123, 90%) expressed at least focal (>1%) TIM-3, and 19% (23/123) showed diffuse (>50%) expression. Diffuse expression was most common in lobular (4/15, 27%), mucinous (9/16, 56%), and medullary-like (3/11, 27%) histologies. In contrast, only 9% (5/55) of conventional ductal, 9% (1/11) of pleomorphic lobular, 10% of apocrine (1/10), no micropapillary (0/1), and no metaplastic carcinomas (0/4) had >50% staining. Diffuse expression was slightly higher in grade 1 when compared to grade 2-3 cancers (26%, 9/34 vs. 14%, 12/87) and in hormone receptor-positive vs. negative tumors (20%, 16/81 vs 14%, 5/37), however these trends were not statistically significant.

Figure 1 – 143

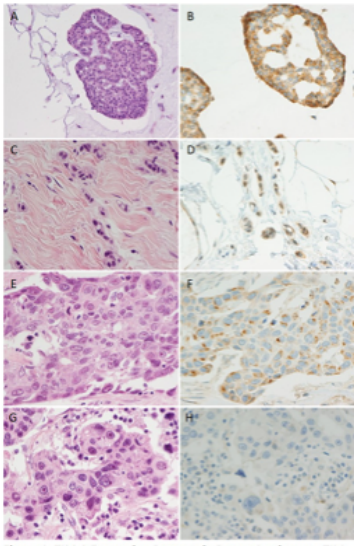

**Figure 1: TIM-3 Expression Patterns in Breast Carcinoma.** TIM-3 was strongly positive in many mucinous (A-B) and lobular (C-D) breast cancers. Some high-grade, triple-negative cancers with medullary-like features were also positive (E-F), while others were entirely negative (G-H).

**Conclusions:** Emerging therapies targeting the checkpoint inhibitor TIM-3 may show efficacy in breast cancer, especially among low-grade, hormone receptor-positive tumors, which can be a clinical challenge with late recurrences and endocrine therapy refractoriness. Diffuse expression is particularly common among lobular and mucinous histologies (see Figure 1), which have previously demonstrated low to absent PD-L1 expression and little response to anti-PD-1/PD-L1 therapies. These results underscore the role for biomarkers in optimizing immunotherapeutic approaches for breast cancer patients, and suggest that checkpoint inhibitors targeting TIM-3 may be useful in women who have not seen benefit from currently available therapies.

## 144 Repeat Biomarker Status in Breast Resection Specimens in Cases with Controlled Cold Ischemic Time

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**Disclosures:** Ellen East: None; Julie Jorns: None

**Background:** Prolonged time from specimen excision to adequate formalin exposure, or cold ischemic time (CIT), negatively impacts estrogen receptor (ER), progesterone receptor (PR) and HER2 biomarker studies routinely performed on breast specimens. Current CAP/ASCO guidelines recommend CIT of ≤1 hour, although some publications support CIT of up to 4 hours. We retrospectively evaluated biomarker status changes from biopsy specimens, which typically are exposed to formalin well within CAP/ASCO guidelines, to resection specimen following implementation of a breast specimen triage protocol designed to optimize CIT.

**Design:** We identified breast resection specimens from 2014-2016 (after institutional implementation of breast specimen triage protocol). Pathology reports were reviewed for clinicopathologic features, CIT, and performance of repeat ER, PR, and/or HER2 studies.

**Results:** 2821 excision specimens from 2344 patients (mean age 56.5, range 24-91) were reviewed, of which 295 specimens had a prior malignant diagnosis (pLCIS, DCIS and/or invasive carcinoma), CIT ≤4 hours and repeated ER, PR and/or HER2 studies. 181 (61.4%) were lumpectomies and 114 (38.6%) mastectomies. Core biopsy was the preceding procedure in 293 (99.3%) and excisional biopsy in 2 (0.7%).

230 (78.0%) had CIT of ≤1 hour and 65 (22.0%) had CIT of >1 hour but ≤4 hours. HER2 was the most commonly repeated study (237, 80.3%), followed by ER, PR and HER2 (48, 16.3%) and ER and/or PR only (10, 3.4%).

250 (84.7%) had biomarker studies repeated based on CAP/ASCO guidelines, for which indications included grade 3, previously HER2 negative tumors (199, 79.6%), HER2 equivocal status on biopsy (42, 16.8%), and small focus of invasion on biopsy (5, 2.0%). Additional indications include absence of internal control on core biopsy, difference in histology from biopsy to resection, and rapid (<1 yr) recurrence after therapy (1, 0.4% each).

ER/PR and HER2 status uncommonly changed. 4 (7%) with ER/PR change had only one marker positive and all were focal (<10%) positive. 5/285 had significant HER2 change, 3 from negative to positive, one of which was by HER2 copy number only, and 2 from positive to negative, one of which had only microinvasive disease on both biopsy and resection (Table 1).

| | | | | |
|---|---|---|---|---|
| Change in ER/PR (N=57) | | | | |
| No | 53 (93%) | | | |
| Yes (biopsy negative, resection low positive) | 1 (1.7%) | Biopsy: Resection:<br>ER (0%) ER (1.2%)<br>PR (0%) PR (0%) | | |
| Yes (biopsy low positive, resection negative) | 3 (5.3%) | Case 1:<br>Biopsy: Resection:<br>ER (0%) ER (0%)<br>PR (5%) PR (0%) | Case 2:<br>Biopsy: Resection:<br>ER (0%) ER (0%)<br>PR (3%) PR (0%) | Case 2:<br>Biopsy: Resection:<br>ER (5%) ER (0%)<br>PR (0%) PR (0%) |
| Change in HER2 (N=285) | | | | |
| No | 248 (87%) | | | |
| Yes (biopsy negative, resection equivocal) | 7 (2.4%)* | | | |
| Yes (biopsy equivocal, resection negative) | 22 (7.7%) | | | |
| Yes (biopsy negative, resection positive) | 3 (1.1%)* | Case 1:<br>Biopsy: Resection:<br>FISH Not Amp IHC (2+)<br>(1.7/3.85) FISH Amp<br>(2.64/4.1) | Case 2:<br>Biopsy: Resection:<br>IHC (1+) IHC (2+)<br>FISH Amp<br>(1.57/6.8) | Case 3:<br>Biopsy: Resection:<br>IHC (1+) IHC (2+)<br>FISH Amp<br>(2.10/6.0) |
| Yes (biopsy equivocal, resection positive) | 3 (1.1%) | | | |
| Yes (biopsy positive, resection negative) | 2 (0.7%) | ^Case 1:<br>Biopsy: Resection:<br>IHC 3+ IHC (2+)<br>FISH Not Amp<br>(1.27/2.0) | Case 2:<br>Biopsy: Resection:<br>FISH Amp IHC (2+)<br>(2.29/6.3) FISH Not Amp<br>(1.27/3.6) | |

*All (10) done for grade 3, previously HER2 NEG CAP/ASCO reason.

^Microinvasive carcinoma on both biopsy and resection.

NA, non-amplified; Amp, amplified (HER2/CEP 17 ratio/Average HER2 copies/cell)

**Conclusions:** When CIT is optimized, biomarker status uncommonly changes in a clinically meaningful way, in this study changing 7% from ER/PR focal (<10%) positive to negative or vice versa and 1.8% HER2 positive to negative or vice versa.

## 145 Encapsulated and Solid Papillary Carcinomas of the Breast with High-grade Features: A Clinicopathological Analysis of 13 Cases

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**Disclosures:** Ellen East: None; Celina Kleer: None; Andrew Sciallis: None

**Background:** Papillary carcinoma (PC) of the breast is a subtype of breast cancer characterized by a proliferation of malignant epithelial cells in association with fibrovascular cores. Encapsulated papillary carcinoma (EPC) and solid papillary carcinoma (SPC) represent two subtypes of PC. In pure form, EPC and SPC have a favorable clinical course compared to conventional invasive breast carcinoma. In most cases, EPC and SPC are comprised of cells with low-to-intermediate grade nuclei, however, rare cases with high-grade features, such as high-grade nuclei and increased mitoses, have been observed. We present a series of PC with high-grade features.

**Design:** We searched our archives for cases of EPC or SPC with high-grade features: cases composed of cells with increased nuclear atypia (i.e. Nottingham score 2 or 3) and mitotic activity (i.e. Nottingham score 2 or 3). All available H&E and immunostained slides were examined. Cases were determined to have (1) circumscribed/pushing/intracystic growth, (2) jigsaw-like pattern of stromal fibroplasia, or (3) infiltrative growth (i.e. unequivocal stromal invasion or a conventionally invasive component). Tumor size, necrosis, lymph-vascular space involvement, unusual components (e.g. micropapillary or metaplastic differentiation), and relevant clinical parameters were also recorded.

**Results:** Our search yielded 13 cases:12 female and 1 male (average age 64 years). Average tumor size was 1.8 cm (range 0.6-3.2). Most cases had severe cytologic atypia (grade 3 in 11 cases) and increased mitoses (average 24 per 10 hpf, range 6-55). Necrosis and LVI were present in 10 (77%) and 0 cases, respectively. Infiltrative growth was seen in 7 cases (54%), pushing in 3 (23%), and jigsaw-like in 3 (23%). Prior core biopsy showed clear infiltrative growth in only 5 cases. Triple-negative status was seen in 5 cases (38%; 1 with metaplastic differentiation); 0 were HER2-positive.

Lymph node metastasis was seen in 2 patients (1 jigsaw-like, 1 infiltrative); 2 different patients had recurrence (1 jigsaw-like, 1 infiltrative). No patient died of disease (average length of follow-up 34 months; range 4-92).

**Conclusions:** Most high-grade PCs had worrisome growth patterns, either infiltrative or jigsaw-like, and a significant subset experienced recurrence or lymph node metastasis. These results support previous observations that high-grade PC may behave similar to conventional invasive breast carcinoma.

## 146 Comparing Oncotype DX and IHC4 Recurrence Risk Scores When Performed on the Same versus Different Tissue Blocks

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**Disclosures:** Jake Eggett: None; Melissa Wright: None; Margaret VanMeter: None; Dylan Miller: None

**Background:** Utilization of molecular testing for breast cancer recurrence risk prediction can be optimized by reporting immunohistochemistry-based (IHC4) recurrence risk algorithm scores. Several studies have shown good agreement between scores obtained by these algorithms and the Oncotype DX (ODX) recurrence score. It is not clear how testing different specimens (eg IHC4 on core biopsy versus ODX on mastectomy) from the same patient influences agreement between the two scores. We report a comparison of IHC4 and ODX testing using the same and different tissue blocks.

**Design:** Both routine breast prognostic factor testing (ER, PR, HER2, and Ki67) with the 'Magee 3' IHC4 algorithm and ODX testing were performed on invasive breast carcinomas. By convention in our lab, IHC4 testing is performed on core biopsy material primarily. ODX testing is typically ordered after definitive surgery and performed on excisional tumor samples. For various reasons, IHC4 and ODX are occasionally performed on the same block. Descriptive and correlative statistical comparisons were made between IHC4 and ODX scores when the same block versus different blocks were tested.

**Results:** Among 484 invasive breast cancers tested with both IHC4 and ODX, 94 were performed on the same tissue block and 390 on different tumor blocks. Clinical and pathologic features between the 2 groups were similar. Same block testing was performed on 54 core biopsies and 40 excisional samples. For the other 390 cases, IHC4 was performed on a core biopsy and ODX on excisions. Overall agreement between IHC4 and ODX was good and no difference in the degree of agreement (i.e. the absolute Δ between the 2 scores) was seen whether the same or different blocks were tested (p=0.09).

| | Same Block Tested | | Different Block Tested | | | |
|---|---|---|---|---|---|---|
| | **Mean** | **Range** | **Mean** | **Range** | | |
| Patient Age | 53.7 | 34-73 | 55.7 | 30-76 | | |
| ODX Score | 18.2 | 0-75 | 19.2 | 0-76 | | |
| IHC4 Score | 20.5 | 10-42 | 19.4 | 10-42 | | |
| \|Δ ODX - IHC4\| | **6.9** | **0-34** | **5.8** | **0-35** | **p=0.09** | |
| | **n=** | **%** | **n=** | **%** | | |
| ODX ≤25, IHC4 ≤25 | 61 | **65** | 294 | **75** | p=NS | |
| ODX ≤25, IHC4 >25 | 8 | **9** | 21 | **5** | p=NS | |
| ODX >25, IHC4 ≤25 | 7 | **7** | 35 | **9** | p=NS | |
| ODX >25, IHC4 >25 | 18 | **19** | 40 | **10** | p=NS | |

**Conclusions:** Concordance between IHC4 and ODX was high (85%) and remained so whether the test was performed on the identical block or on different samples from a given tumor. This suggests that despite technical and interpretive differences, each testing method is robust with respect to the potential confounding effects of tumor sampling, biopsy site reaction and other factors.

## 147 Inflammatory Markers Expression in Normal Breast Tissue and Breast Cancer Prognostic Factors

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**Disclosures:**Kaoutar Ennour-Idrissi: None; Caroline Diorio: None

**Background:** Although chronic sustained inflammation has been shown to predispose to different forms of cancer, the role of specific inflammatory cytokines is not completely understood and their implication in breast cancer natural history still controversial. Studies of the relationship between circulating inflammatory markers and breast cancer prognosis have yielded inconsistent results, and none had measured breast tissue local inflammation. The objective of the present study was thus to examine the association between inflammatory markers expression in normal breast tissue and breast cancer prognostic factors.

**Design:** Data were prospectively collected at time of surgery from 113 breast cancer patients recruited consecutively between 2011 and 2012, at a breast cancer reference center. Expression level of 11 inflammatory markers was measured by immunohistochemistry in normal breast tissue. Tumor biological characteristics were extracted from pathology reports. Adjusted spearman correlations and multivariate log-binomial models were used to estimate the associations between inflammatory markers expression and breast cancer prognostic factors.

**Results:** Higher level of stromal expression of COX2 was correlated with higher disease stage ($r_s$=0.28, *p=0.005*), higher number of involved lymph nodes ($r_s$=0.29, *p=0.003*), and positive PR status ($r_s$=0.25, *p=0.013*). Higher lactoferrin expression was correlated with positive HER2 status ($r_s$=0.34, *p=0.0007*) and higher tumor grade ($r_s$=0.25, *p=0.013*), whereas higher TGF-beta expression was correlated with smaller tumor size ($r_s$=-0.22, *p=0.0311*). Higher stromal expression of COX2 was associated with higher disease stage (RR=1.13 [1.03 ; 1.24], *p=0.011*), positive PR status (RR=1.02 [1.01 ; 1.03], *p=0.007*) and lymph node metastasis (RR=1.29 [1.13 ; 1.48], *p=0.0002*). Higher lactoferrin expression was associated with positive HER2 status (RR=1.07 [1.03 ; 1.11], *p=0.0001*) and higher TGF-beta expression was associated with smaller tumor size (RR=0.83 [0.75 ; 0.93], *p=0.0008*).

**Conclusions:** Local inflammation in normal breast seems to be related to breast cancer invasiveness. Higher levels of two inflammatory cytokines (lactoferrin and stromal COX2) were associated with higher breast cancer invasiveness whereas higher levels of one anti-inflammatory marker (TGF-beta) were associated with less aggressive tumors. These findings indicate a potential benefit from anti-inflammatory drugs for breast cancer prevention and treatment.

## 148 The Impact of 2018 ASCO-CAP HER2 Testing Guidelines on Breast Cancer HER2 Results. An audit of 2136 consecutive cases evaluated by Immunohistochemistry and In Situ Hybridization

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**Disclosures:** Gelareh Farshid: None; Deepak Dhatrak: None; Amardeep Gilhotra: None; Barbara Koszyca: None; James Nolan: None

**Background:** The 2018 iteration of the ASCO-CAP HER2 testing guidelines has modified the 2013 criteria in a number of areas, including the definition of 2+ IHC, discretionary re-testing of resections after a negative result on core biopsy, enumeration of five clinical groupings of HER2 test results and emphasis on integration of concurrent IHC and ISH results for cases in groups 2-4. In these guidelines, there are no circumstances in which cancers with 0 or 1+ IHC are considered HER2 positive and apart from group 3 lesions, 3+ IHC reactivity is required for a positive HER2 result. We wished to evaluate the impact of these guidelines on clinical practice.

**Design:** After approval by IRB, we applied the 2018 HER2 testing criteria to a series of consecutive invasive breast cancers evaluated by immunohistochemistry and in situ hybridization at our laboratory based on 2013 criteria, as part of routine clinical practice. Our primary

objective was to determine the impact of the new guidelines on HER2 positive rates. Secondary objectives included correlations between HER2 IHC and ISH results and in assessing the clinical profiles of cancers in each of the HER2 subgroups.

**Results:** During January 2012 to February 2017, 2316 consecutive samples of invasive breast carcinoma were evaluated for biomarkers, cores in 31.2%, resections in 68.2% and cell blocks in 7 cases (0.03%). IHC results are available in 2109 cases, dual probe ISH in 1827 cases and single probe in 309 cases. The distribution of ISH groups is: Group 1: 16.5%, 2: 1.0%, 3: 0.3%, 4: 2.6% and 5: 79.5%. Table 1 shows the distribution of HER2 groups and the correlation with age, grade, ER and PR. In 99.01%, the 2013 and 2018 results were concordant. The results were discordant in 21 cases (0.99%) all in groups 2-4, 11 cases previously classified as amplified, were negative by 2018 criteria, the reverse was true for 9 cases, while 1 case, originally indeterminate due to unsatisfactory ISH, is now classified as negative, owing to 1+ IHC. Based on 2018 criteria, the HER2 positivity rate by IHC score was: 0: (1/582) 0.17%, 1+: (5/785) 0.63%, 2+: (40/437) 9.15%, 3+: (275/295) 93.22%, unassessable: (2/7) 28.57%.

| Clinical Features | 1. Classic amplified | 2. Monosomy | 3. Co-amplified | 4. Borderline | 5. Classic Not amplified |
|---|---|---|---|---|---|
| N | 301 | 19 | 6 | 48 | 1244 |
| Proportion (%) | 16.5 | 1.04 | 0.33 | 2.63 | 79.5 |
| Mean age (yrs) | 57.9 | 64.4 | 50.3 | 62.8 | 62.7 |
| Grade 1 (%) | 1.1 | 12.5 | 0 | 0 | 19.9 |
| Grade 2 (%) | 17.6 | 18.7 | 0 | 23.7 | 45.1 |
| Grade 3 (%) | 76.1 | 68.8 | 100 | 76.9 | 35 |
| ER positive (%) | 62.7 | 84.2 | 100 | 84.8 | 88.4 |
| PR positive (%) | 59.3 | 78.9 | 50 | 80.4 | 84.7 |

**Conclusions:** The 2018 ASCO-CAP HER2 testing criteria produce results highly concordant with the 2013 criteria. Groups 2-4 represent 4% of the cases and are the sources of the discordances, split evenly between those now regarded as positive or negative. Concurrent IHC shows 0.44% of cases with IHC of 0 or 1+ were finally classified as positive by ISH.

## 149 Solid Papillary Carcinoma of the Breast: an Institutional Review and Comparison of Conventional and Invasive Patterns

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**Disclosures:** Cameron Felty: None; Christopher Jackson: None; Kristen Muller: None

**Background:** Background: Solid papillary carcinomas (SPC) are rare tumors of the breast that form circumscribed cellular nests often in “geographic” patterns, usually lack myoepithelium, and generally convey a good prognosis. Uncertainty can arise when attempting to classify, and manage, SPC as either in situ or invasive disease. A subset of SPC demonstrate a more infiltrative pattern with large irregular tumor nests often with surrounding desmoplasia, which some consider “invasive SPC”. The primary aim of this study was to compare the clinicopathologic features of conventional and invasive SPC.

**Design:** We performed a retrospective database search for breast surgical excisions containing “papillary carcinoma” from 1/2000 – 8/2018. H&E and myoepithelial IHC slides were independently reviewed by two breast pathologists who classified lesions as conventional SPC or invasive SPC according to WHO criteria. Clinicopathologic features and follow up were compared.

**Results:** Thirty cases of SPC were identified: 15 conventional SPC and 15 invasive SPC; clinicopathologic features are shown in table 1. A coexisting invasive carcinoma (predominantly NOS or mucinous types) was identified in 14 (47%) of all cases, of which seven had lymph node metastases. Interestingly, 3 lymph node metastases resembled the SPC and not the invasive carcinoma. Invasive SPC was found to show more suspicious radiologic findings according to the BIRADS reporting system, tended to be higher grade, larger, and have a higher frequency of axillary lymph node metastases. Comparing SPC (n=8) and invasive SPC (n=8) without associated conventional invasive carcinoma, tumor size and suspicious radiologic findings persisted as notable differences (SPC 0.98 ± 0.4 cm versus invasive SPC 2.0 ± 0.9, p = 0.01; SPC 100% BIRDAS category 4 versus invasive SPC 50% BIRADS category 5, p=0.07). Patients received adjuvant treatment

including radiation therapy, anti-hormonal therapy, and chemotherapy in 55%, 75%, and 10% of cases, respectively. All patients have NED (48.5 ± 39.8 months).

| | SPC (n=15) | Invasive SPC (n=15) | p value |
|---|---|---|---|
| Age (years) | 74.3 ± 9 | 71.6 ± 15 | 0.5 |
| Suspicious radiology (BIRADS 5) | 3/14 (21%) | 9/14 (64%) | 0.05 |
| SPC grade (intermediate or high) | 9/15 (60%) | 15/15 (100%) | 0.016 |
| Conventional invasive present | 7/15 (47%) | 7/15 (47%) | 1.0 |
| Tumor size (cm) | 1.1 ± 0.6 | 3.1 ± 2.4 | 0.004 |
| DCIS present | 13/15 (87%) | 10/15 (67%) | 0.3 |
| LVI present | 1/15 (7%) | 4/15 (27%) | 0.3 |
| Lymph node metastasis | 1/15 (7%) | 6/14 (43%) | 0.09 |
| ER positive | 15/15 (100%) | 14/15 (93%) | 1.0 |
| HER2 negative | 11/11 (100%) | 14/14 (100%) | 1.0 |

**Conclusions:** SPCs contain a spectrum of morphologic patterns; however, in our cohort, when compared to the conventional pattern, SPCs with an invasive pattern were associated with potentially less favorable features, including higher tumor grade, larger size, and more frequent lymph node metastases.

## 150 p53 Expression in Young Women with Breast Cancer

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**Disclosures:** Brian Finkelman: None; Jennifer Pincus: None; K. P. Siziopikou: None; Luis Blanco: None

**Background:** *TP53* is the one of the most commonly mutated genes in breast cancer, with mutations seen in 20-30% of cases overall and at higher rates in specific subtypes and with less favorable histology, such as triple-negative and grade 3 tumors. p53 expression, as assessed by immunohistochemistry (IHC), is often used as a surrogate of *TP53* mutation status. Although younger women are more likely than older women to have germline mutations in *TP53* and more aggressive breast cancer subtypes, less is known about breast cancer p53 expression in young women. We examined p53 expression and its relationship to tumor subtype in a cohort of young women with breast cancer at our academic institution.

**Design:** All cases of invasive breast cancer at our institution in women <40 years of age at surgery between January 1, 2009, and June 4, 2018, who had p53 IHC testing performed as part of routine clinical care, were included. Tumor subtypes were defined using IHC: ER+/HER2-/Ki-67≤20% for luminal-A; ER+/HER2+ or ER+/HER2-/Ki-67>20% for luminal-B; ER-/HER2+ for HER2-like; and ER-/PR-/HER2- for triple-negative. Histopathologic features assessed included tumor size, grade, histologic type, presence of lymphovascular invasion (LVI), and lymph node status. Rates of p53 expression were assessed in the overall cohort as well as stratified by tumor subtype.

**Results:** 404 breast cancer patients were identified (age range 17-39), most with ductal histology (374/404, 93%). p53 expression was observed in 117/404 (29%) of cases overall, with higher rates seen with grade 3 histology (86/189, 46%, P<0.001), but not with T3 stage, presence of LVI, or positive lymph nodes. Among the 344 patients who could be classified into tumor subtypes, p53 expression was significantly different across subtypes (P<0.001), with p53 expression observed in 16/152 (11%) of luminal-A, 34/114 (30%) of luminal-B, 7/15 (47%) of HER2-like, and 37/63 (59%) of triple-negative tumors.

**Conclusions:** Our study is one of the largest to date to examine p53 expression and its relationship to tumor subtypes in young women with breast cancer. Despite high rates of aggressive subtypes and grade 3 tumors, our cohort of young patients showed an overall rate of p53 expression that was similar to the reported *TP53* mutation rate among all breast cancer cases in the literature. Further molecular studies are needed to determine whether breast carcinomas in young women may be more likely to harbor specific *TP53* mutations that may be less readily identified by IHC.

## 151 Breast Cancer Subtypes and Histopathologic Features in Very Young Women

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

**Background:** About 7% of breast cancer cases in the US are in women <40 years of age, and these patients tend to have more aggressive cancer subtypes, less favorable histopathologic profiles, and poorer outcomes. However, little is known on whether young women themselves vary by age in terms of breast cancer subtypes and histopathologic features. In this study, we compare such features among young (age 30-39) and very young (age ≤30) women with breast cancer at our academic institution.

**Design:** All invasive breast cancer cases at our institution in women age <40 at surgery between January 1, 2009, and June 4, 2018, were included. Tumor subtypes were defined using immunohistochemical markers: ER+/HER2-/Ki-67≤20% for luminal-A; ER+/HER2+ or ER+/HER2-/Ki-67>20% for luminal-B; ER-/HER2+ for HER2-like; and ER-/PR-/HER2- for triple-negative. Cancer subtype rates were compared to those in women age ≥50, based on national SEER data (N=39,926). Tumor size, grade, histologic type, presence of lymphovascular invasion (LVI), and lymph node status were assessed.

**Results:** 388 breast cancer patients were identified (age range 17-39), including 51 very young (age ≤30) and 337 young (age 31-39) women. Nearly all very young patients had ductal histology (98%). 63% had a T1 (≤2 cm) and 31% had a T2 (2-5 cm) lesion. Additionally, 45% were grade 3, 39% had lymphovascular invasion, and 37% had positive lymph nodes. Almost half of the tumors in very young women were luminal-B, significantly more than in young women (47% vs 30%, P=0.015). Rates of luminal-A (33% vs 40%), HER2-like (4% vs 7%), and triple-negative (16% vs 23%) tumors, as well as all other histopathologic features, were similar across groups. Compared to women age ≥50, very young women had lower rates of luminal-A (33% vs 75%, P<0.001) and higher rates of luminal-B (47% vs 9%, P<0.001) tumors, while there was no difference in the rates of HER2-like and triple-negative tumors.

**Conclusions:** Our study is one of the largest to date to examine breast cancer subtypes and histopathologic features in very young women. Very young women were more likely than young women to have luminal-B cancer. In addition, compared to SEER data on women age ≥50, very young women had lower rates of luminal-A and higher rates of luminal-B tumors. These results await confirmation with future molecular studies; however, they suggest that breast cancer subtypes may vary by age in young women, with potential implications for treatment strategies.

## 152 Immunohistochemical Evaluation of Pan-TRK in Secretory Breast Carcinoma

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**Disclosures:** Elizabeth Fowler: None; Beth Harrison: None; Yunn-Yi Chen: None; Gregor Krings: None; Anne Vincent-Salomon: None; Laetitia Fuhrmann: None; Beiyun Chen: None; Elizabeth Hosfield: None; Sandra Barnick: None; Jason Hornick: None; Stuart Schnitt: None

**Background:** Secretory carcinoma (SC) is a rare special type breast cancer underpinned by a recurrent t(12;15)(p13;q25) translocation resulting in *ETV6-NTRK3* gene fusion. While the diagnosis of SC is usually straightforward, some cases pose diagnostic problems; FISH using *ETV6* break apart probes can be used to confirm the diagnosis in challenging cases. More recently, immunohistochemistry (IHC) using a pan-TRK antibody has been shown to aid in identifying NTRK rearrangements in other tumor types, but its sensitivity and specificity for breast SCs has not been evaluated. The purpose of this study was to assess the diagnostic utility of pan-TRK IHC for breast SC, in comparison to other types of breast cancer and histologic mimics.

**Design:** IHC was performed on whole sections of 22 SC and tissue microarray (TMA) sections of SC mimics (n=15) and other breast cancer types of various grades and receptor status (n=208) using a rabbit monoclonal pan-TRK antibody (1:100 dilution; clone EPR17341; Abcam) following antigen retrieval with 1mM EDTA (pH 8.0) in a pressure cooker. Cases were evaluated for the presence, location (nuclear, cytoplasmic, membranous) and intensity (weak, moderate, strong) of staining.

**Results:** Median age and tumor size of the 22 SC patients were 47.5 yrs and 1.1 cm, respectively. *ETV6* FISH was positive in all 18 cases with adequate signals. Among the 22 SC, 20 (90.9%) showed staining for pan-TRK: exclusively nuclear in 18 (90%), primarily nuclear with weak cytoplasmic staining in 1, and primarily cytoplasmic with focal nuclear staining in 1. Among the 18 with primarily nuclear staining, staining intensity was variable, but was at least focally strong in 14. Of the two pan-TRK negative cases, one was a core needle biopsy with very limited tumor and one was an excision with unusual morphology for a SC, but positive for *ETV6* rearrangement by FISH. No pan-TRK staining was seen in histologic mimics (i.e., lactational change and cystic hypersecretory lesions). Among the 208 invasive carcinomas of other types, 21 (10.1%) showed very focal, weak, barely perceptible nuclear staining in <5% of tumor cells and 1 (0.5%) showed very focal membranous staining for pan-TRK.

**Conclusions:** We report for the first time the value of pan-TRK IHC as an adjunct in the diagnosis of breast SC. At least focally strong nuclear staining is a sensitive and specific marker for SC that provides a more rapid and cost-effective method than *ETV6* FISH to confirm the diagnosis in problematic cases.

## 153 ID Proteins are Key Regulators of the Epithelial-Mesenchymal Transition in Breast Cancer Subtypes

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**Background:** Epithelial to mesenchymal transition (EMT) is a key process during tumor development consisting in changes in cell polarity, morphology and interactions leading to the acquisition of migration ability. As a result, tumor cells spread to the bloodstream causing distant metastasis. Inhibitor of DNA binding (ID) proteins, a family of four bHLH transcriptor factors, have been shown an essential role in tumor growth and colonization of distant organs after breast cancer (BC) metastasis. Our aim was to assess whether EMT mechanisms in BC are somehow driven by ID proteins.

**Design:** We generated a cellular model for the study of EMT. We cultured 5 cell lines representing all BC phenotypes in monolayer. Cells were incubated at 37ºC in a humidified 5% $CO_2$ air atmosphere in DMEM media supplemented with 10% fetal bovine serum, 1% Glutamine, 50 U/ml of penicillin and 50 mg/ml of streptomycin. Further, cells were transferred to non-adherent plates and cultured with Mammocult Media to generate tumor mammospheres, mimicking the mesenchymal state. We extracted mRNA of each condition (epithelial-like and mesenchymal-like) and analyzed gene expression (*ID1-4, SNAIL1, TWIST1* and *VIMENTIN,* with *PUM1* and *β-ACTIN* as reference genes*)* by qRT-PCR using TaqMan® Assays. Three biological replicates were included and all experiments were done twice. Relative changes in gene expression were calculated as the fold change by the $2^{-\Delta\Delta Ct}$ method. Overexpression was defined as >150% increased levels compared to the control.

**Results:** We successfully generated tumor mammospheres that were able to grow detached from the plate. Compared with BC cell lines growing in monolayer, mammospheres overexpressed at least one of the mesenchymal markers tested: *SNAIL1* (all cell lines), *VIMENTIN* (BT474 and MDA-MB231) or *TWIST1* (MCF7, T47-D). Interestingly, all mammospheres but the ones generated by MCF-7 (Luminal A) had at least a 50% decreased expression in *ID1/ID3.* Similarly, *ID2/ID4*appeared to be downregulated in 4 out of 5 mammospheres lines (all but T47-D), but at lesser levels.

**Conclusions:** Our model in BC cell lines and derivate mammospheres mimicking the EMT shows that, downregulation of ID gene expression, especially *ID1/ID3*, seems to be essential to achieve the mesenchymal state. Therefore, they might be involved in the progression of the most aggressive subtypes of BC, excluding Luminal A, known to have better clinical behaviour.

## 154 CtBP2 Overexpression in Neoadjuvantly Treated Breast Cancer by Immunohistochemistry and Its Relation to Clinical Outcomes

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**Disclosures:** Matthew Gayhart: None; Kelly McCoy: None; Lorraine Colon Cartagena: None; Michael Idowu: None

**Background:** C-terminal binding proteins 1 and 2 (CtBP1 and CtBP2) are paralogous transcriptional co-regulators which have been implicated in oncogenesis of several solid tumors. Overexpression of CtBP2 is associated poor outcomes in many solid tumors, including breast cancer, and may potentially be an immunotherapy drug target. To our knowledge, CtBP2 overexpression has not been investigated in post neoadjuvant residual tumor to determine its association to outcome. To this end, we examined the association of overexpression of CtBP2 by immunohistochemistry (IHC) in neoadjuvantly treated breast cancer to clinical outcomes.

**Design:** Neoadjuvantly treated patients with residual tumors were selected from a database from 1997-2016. Clinicopathologic, demographic, and follow-up information were obtained. The follow-up period ranges from 9 to 140 months. Patient cases with residual tumors greater than 5 mm were identified and used to create tissue microarrays (TMAs) consisting of triplicate cores using a Beecher ATA-27 arrayer. IHC was performed for CtBP2 (BD, clone16) using a Ventana Benchmark XT autostainer. CtBP2 expression was scored using

the Allred scoring system measuring proportion (1-5) and intensity (1-3). An Allred score greater than 6 was considered overexpression. A statistical analysis was performed using the z-test for two population proportions.

**Results:** A statistically significant relationship was found showing increased survival with respect to all-cause mortality within 5 years of the date of diagnosis in patients with Allred Scores for CtBP2 equal to or less than 6 compared to patients with Allred scores greater than 6 (15.4% vs 40.0%; p-value 0.4363). This relationship continued to be statistically significant when looking at all-cause mortality within 10 years of the date of diagnosis (30.8% vs 55.3%; p-value .04947). No statistically significant relationship was found between the difference in Allred scores and race, stage, and recurrence.

| Allred Score | Number of Cases | 5 Year All-Cause Mortality from Diagnosis | 10 Year All-Cause Mortality from Diagnosis |
|---|---|---|---|
| **?6** | 13 | 2 (15.4%) | 4 (30.8%) |
| **>6** | 85 | 34 (40.0%) | 47 (55.3%) |
| **Total** | 98 | 36 (36.7%) | 51 (52.0%) |

**Conclusions:** Patient's with Allred scores greater than 6 had higher all-cause mortality than patients with Allred scores less than or equal to 6. These results suggest that overexpression of CtBP2 is associated with poor outcomes in in neoadjuvantly treated patients. Additionally, this study also confirms that IHC is able to reveal a clinically significant overexpression of CtBP2 in breast cancer. Further studies with larger patient sample sizes will be useful to confirm the clinical significance of overexpression CtBP2 in NAT Patients.

## 155 What is an upgrade rate for classic lobular neoplasia diagnosed on core needle biopsy? A retrospective multi-center study

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**Disclosures:** Iskender Genco: None; Qing Chang: None; Sabina Hajiyeva: None

**Background:** Management of classic lobular neoplasia (cLN) [atypical lobular hyperplasia (ALH) or classic lobular carcinoma in situ (cLCIS)] diagnosed on core needle biopsy (CNB) is still controversial. The ongoing dispute on the management of cLN still leads many patients to be referred for surgical excision in many institutions including ours. Our aim in this study is to review cases of cLN diagnosed on core biopsy to determine the rate and risk factors of upgrade (ductal carcinoma in situ (DCIS) or invasive carcinoma) on excision and to evaluate the necessity of surgery.

**Design:** All breast CNBs with a diagnosis of ALH or LCIS from three different institutions within single health care system between 2013 and 2018 were retrieved. Cases with any additional higher risk lesions (non-classic LCIS, atypical ductal hyperplasia, flat epithelial atypia, DCIS or invasive carcinoma) diagnosed on the same biopsy core, were excluded. Information about age, history/concurrent breast cancer (H/CBC) and excision diagnosis, if existed, was recorded for each patient. Radiologic findings were reviewed to determine the radiologic-pathologic concordance.

**Results:** A total of 769 CNBs from 743 patients had a diagnosis of LN. After excluding 244 biopsies due to having any additional higher risk lesions on same biopsy, 525 isolated cLN cases including 321 ALH and 204 cLCIS were identified. 290 of 525 cases had excision information. All surgically excised cases showed radiologic-pathologic concordance on prior biopsy. Further analysis of 290 excised cLN cases showed 14 (4.8%) upgrade lesions on excision. There were 3 (1.7%) upgrades in 172 ALH cases, which were all invasive lobular carcinomas (ILC). On the other hand, 11 (9.3%) of 118 cLCIS cases revealed upgrade (3 ILC, 7 DCIS and 1 DCIS+ILC). Moreover, cLN cases with H/CBC showed a higher rate of upgrade comparing to cases with no H/CBC. When we analyzed this relationship in ALH and cLCIS cases separately, the relationship was still statistically significant for cLCIS cases but not for ALH cases. Additionally, categorizing the patients as <50 years old and ?50 years old revealed no difference in upgrade rate between two groups.

Figure 1 – 155

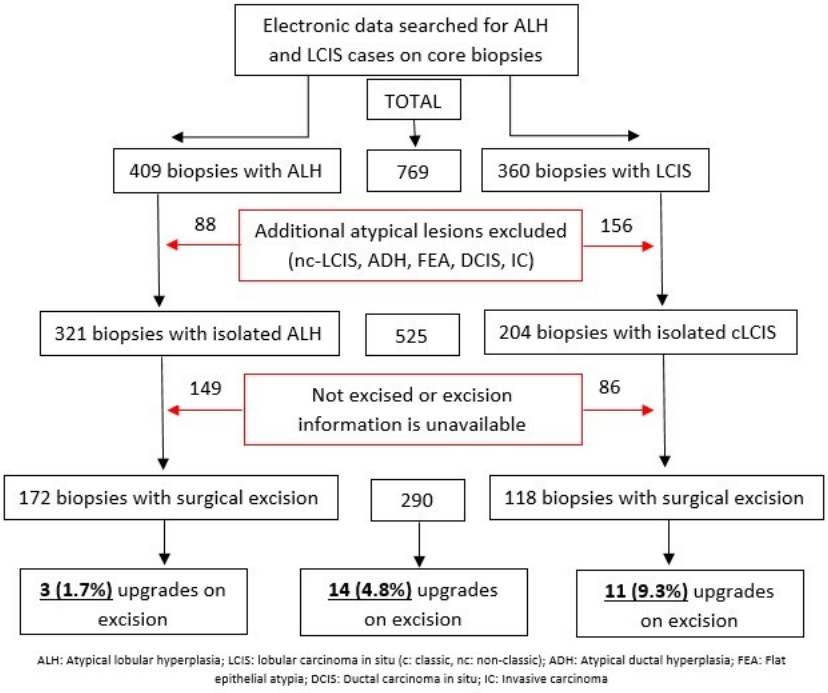


Figure 2 – 155

| | ALH | | | cLCIS | | | cLN | | |
|---|---|---|---|---|---|---|---|---|---|
| | Upgrade | No Upgrade | p | Upgrade | No Upgrade | p | Upgrade | No Upgrade | p |
| *<50* | 1 *(3.6%)* | 27 | 0.42 | 2 *(9.5%)* | 19 | 0.97 | 3 *(6.1%)* | 46 | 0.64 |
| *>50* | 2 *(1.4%)* | 142 | | 9 *(9.3%)* | 88 | | 11 *(4.6%)* | 230 | |
| *H/CBC+* | 1 *(3%)* | 33 | 0.55 | 6 *(21%)* | 23 | ***0.01*** | 7 *(11.1%)* | 56 | ***0,008*** |
| *H/CBC-* | 2 *(1.44%)* | 136 | | 5 *(5.6%)* | 84 | | 7 *(3%)* | 220 | |
| *TOTAL* | 3 *(1.7%)* | 169 | | 11 *(9.3%)* | 107 | | 14 *(4.8%)* | 276 | |

**Conclusions:** Our findings revealed a low upgrade rate of cLN diagnosed on CNB regardless of age. Our data suggest that in cases with radiologic-pathologic correlation, patients with ALH regardless of history/concurrent breast cancer as well as patients with cLCIS without history/concurrent breast cancer can be spared excision.

## 156 Upgrade rate of intraductal papilloma without atypia on core biopsy: A clinical, pathologic and radiologic correlation study

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**Disclosures:** Iskender Genco: None; Sabina Hajiyeva: None

**Background:** A wide range of reported upgrade rates (0% - 28.6%) on surgical excision of benign intraductal papilloma (IDP) diagnosed on breast core needle biopsy (CNB) makes management of IDP controversial. However, some studies which reported high upgrade rates included cases showing radiologic-pathologic discordance or additional atypical lesions on the same CNB that might also lead to upgrade on excision.

**Design:** We electronically searched our pathology department database for breast CNB with diagnosis of IDP from 2013 to 2018. The cases which had radiologic-pathologic discordance, any type of atypia on the same CNB, no prior biopsy or excision slides avialable for our review, or no excision information were excluded. All slides of CNBs and surgical excisions were reviewed by two pathologist and assesed for size of IDP, fragmentation and calcification on CNBs as well as for presence of remaining IDP or any other atypical lesion on excision. Clinical (age, history of breast cancer) and radiologic features (mode of biopsy, size of lesion, distance from nipple) were also noted.

**Results:** After applying the exclusion criteria, 126 IDP cases from 94 patients were identified and included in the study for further analysis. The mean age of the patients was 54 years (29-84) and 14 patients had prior or concurrent breast cancer. The majority of cases (71%) were diagnosed on ultrasound-guided CNB. The upgrade rate was 1.58% (2/126). Both upgrade cases were ductal carcinoma in situ (DCIS) with low and intermediate nuclear grade. The distances between DCIS and residual IDP were 3 mm and 8 mm. Additionally, one patient with upgrade had concurrent invasive carcinoma in the contralateral breast. None of the clinical, radiologic or histopathologic features significantly predicted the upgrade lesions on excision.

Figure 1 - 156

**Clinical, Radiologic and Histopathologic Characteristics of Patients According to Upgrade on Excision**

| | TOTAL | No Upgrade | Upgrade | *p value* |
|---|---|---|---|---|
| **IDP on Core Biopsy** | 126 (100%) | 124 (98.4%) | 2 (1.58%) | |
| **Age** | | | | |
| < 50 years old | 59 | 59 | 0 | *0,498* |
| > 50 years old | 67 | 65 | 2 | |
| **Personal History of Breast Cancer** | | | | |
| Yes | 14 | 13 | 1 | *0,211* |
| No | 112 | 111 | 1 | |
| *Concurrent* | | | | |
| Yes | 7 | 6 | 1 | *0,123* |
| No | 119 | 118 | 1 | |
| *Prior* | | | | |
| Yes | 7 | 7 | 0 | *1,000* |
| No | 119 | 119 | 0 | |
| **Radiology** | | | | |
| *Mode of biopsy* | | | | |
| US-guided | 90 | 88 | 2 | *1,000* |
| MRI-guided | 22 | 22 | 0 | |
| Stereotactic | 14 | 14 | 0 | |
| *Size* | | | | |
| <10 mm | 105 | 104 | 1 | *0,307* |
| >10 mm | 21 | 20 | 1 | |
| *Distance from nipple* | | | | |
| <20 mm | 30 | 29 | 1 | *0,149* |
| >20 mm | 96 | 95 | 1 | |
| **Pathology** | | | | |
| *Size* | | | | |
| <2 mm | 16 | 16 | 0 | *1,000* |
| >2 mm | 110 | 108 | 2 | |
| *Fragmentation* | | | | |
| Yes | 40 | 38 | 2 | *0,099* |
| No | 86 | 86 | 0 | |
| *Calcifications* | | | | |
| Yes | 11 | 10 | 1 | *0,168* |
| No | 115 | 114 | 1 | |
| *Presence of Remaining IDP on Excision* | | | | |
| Yes | 95 | 93 | 2 | *1,000* |
| No | 31 | 31 | 0 | |

**Conclusions:** Our findings show a very low upgrade rate (1.58%) on excision of benign IDP diagnosed on CNB with radiologic-pathologic concordance. These findings suggest that non-surgical management of petients with radiologic-pathologic concordant IDP diagnosed on CNB would be appropriate in routine practice.

## 157 Serous-Like Breast Carcinoma: A Novel Subtype of Triple-Negative Breast Cancer

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**Disclosures:** Toshi Ghosh: None; Matthew Goetz: *Advisory Board Member*, Biovica, Novartis, Sermonix; *Grant or Research Support*, Lilly, Pfizer; Daniel Visscher: None

**Background:** Triple-negative breast cancer (TNBC) is morphologically heterogeneous and includes distinctive subsets such as metaplastic and medullary carcinomas. However, histopathological subclassification of TNBC has yet to be fully defined. This study proposes a distinct subtype of TNBC with "serous-like" morphology.

**Design:** Clinicopathological characteristics of 21 cases of "serous-like" TNBC were studied in women who underwent breast surgery at a single institution between 1988 and 2013. These tumors demonstrated high-grade atypia, eosinophilic cytoplasm with occasional columnar cell change, desmoplastic stroma, readily identifiable and atypical mitoses, and apoptotic bodies. They were distinctively arranged in branching and curving glands, with primitive pseudopapillary or micropapillary projections. (Figures 1 and 2). None had concurrent gynecological serous carcinomas.

**Results:** All tumors were triple-negative (ER/PR <1%, HER2-negative) and Nottingham Grade II-III. Median age at diagnosis was 48 years (range, 31 - 83 years), and median follow-up interval was 4.2 years (range, 23 days - 25.5 years). Approximately half had T1 (11/21, 52%) or N0 (10/21, 48%) disease at presentation. 1 of 2 patients who underwent genetic testing had a pathogenic mutation (*BRCA1*). Most with known adjuvant or neoadjuvant treatment received either both chemotherapy and radiation or chemotherapy alone (15/21, 71%). Approximately half had no recurrence since surgery (11/21, 52%), while one-third died of disease (7/21, 33%), and few were alive with local recurrence or distant metastasis (2/21, 10%). 6/21 (29%) developed non-axillary metastasis, 1/21 (5%) developed local recurrence, and 1/21 (5%) developed both local recurrence and distant metastasis. In those alive with disease (n=2), median time to recurrence/metastasis was 9 years (range, 4.8 - 13.2 years). In those who died of disease with known recurrence/metastasis (n=6), median time to recurrence/metastasis was 2.6 years (range, 0.4 - 9.4 years). There was no correlation between outcome and stage or ki-67. (Table 1).

| | | | Local recurrence within each category | Non-axillary metastasis within each category |
|---|---|---|---|---|
| **Age** | **< 50 years** | 11 (52.4%) | 2 (18.2%)* | 3 (27.3%)* |
| | **>/= 50 years** | 10 (47.6%) | 0 | 4 (40.0%) |
| **Tumor size** | **T1 (** | 11 (52.4%) | 1 (9.1%) | 3 (27.3%) |
| | **T2 (21-50 mm)** | 7 (33.3%) | 1 (14.3%)* | 3 (42.9%)* |
| | **T3 (> 50 mm)** | 0 | 0 | 0 |
| | **T4 (chest wall/skin)** | 0 | 0 | 0 |
| | **Unknown** | 3 (14.3%) | 1 (33.3%) | 1 (33.3%) |
| **Axillary nodal status** | **N0 (0)** | 10 (47.6%) | 0 | 2 (20.0%) |
| | **N1 (1-3)** | 4 (19.0%) | 1 (25.0%)* | 2 (50.0%)* |
| | **N2 (4-9)** | 2 (9.5%) | 0 | 1 (50.0%) |
| | **N3 (>/= 10)** | 0 | 0 | 0 |
| | **Unknown** | 5 (23.8%) | 0 | 2 (40.0%) |
| **Adjuvant/neoadjuvant therapy** | **Adjuvant chemotherapy and radiation** | 10 (47.6%) | 2 (20.0%)* | 1 (10.0%)* |
| | **Adjuvant chemotherapy only** | 4 (19.0%) | 0 | 1 (25.0%) |
| | **Neoadjuvant chemotherapy, adjuvant radiation** | 1 (4.8%) | 0 | 1 (100.0%) |
| | **None** | 3 (14.3%) | 0 | 3 (100.0%) |
| | **Unknown** | 3 (14.3%) | 0 | 1 (33.3%) |
| **Outcome** | **No recurrence since surgery** | 11 (52.4%) | - | - |
| | **Died of disease** | 7 (33.3%) | 1 (14.3%)* | 6 (85.7%)* |
| | **Alive with local recurrence or distant metastasis** | 2 (9.5%) | 1 (50.0%) | 1 (50.0%) |
| | **Died of unrelated disease** | 1 (4.8%) | 0 | 0 |
| *One patient developed concurrent local recurrence and non-axillary metastasis | | | | |

Figure 1 - 157

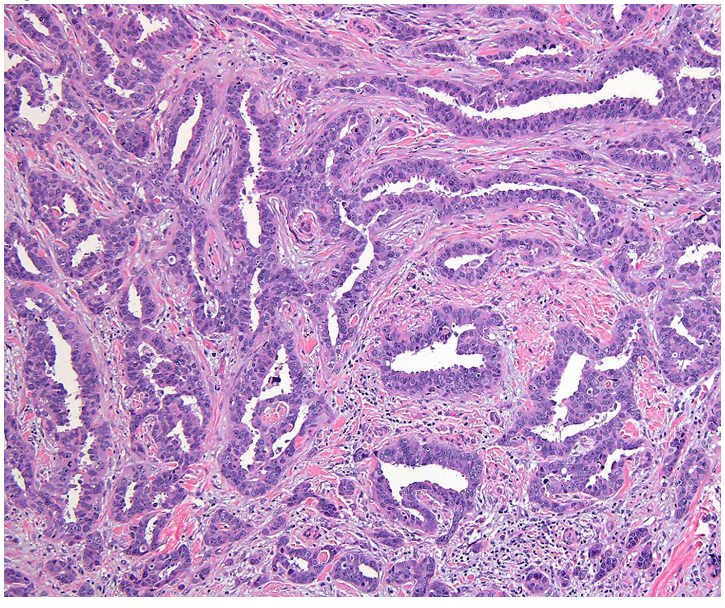
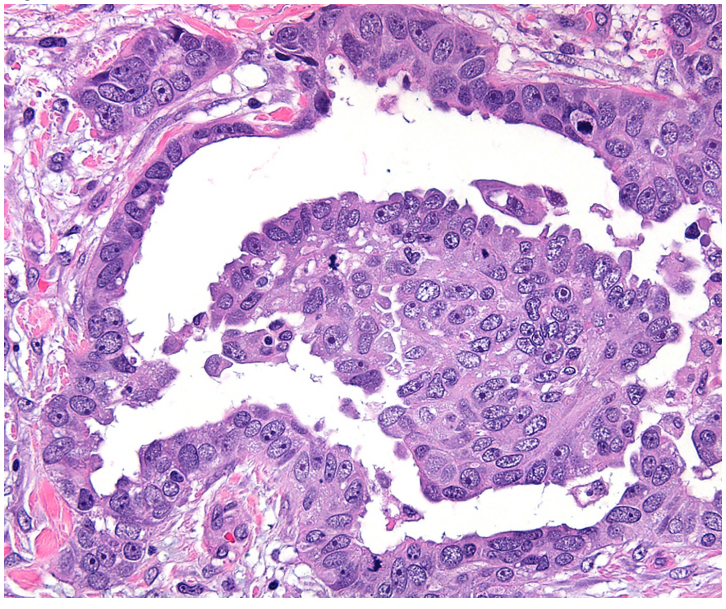


Figure 2 - 157



**Conclusions:** Serous-like breast carcinoma represents a unique subtype of TNBC with characteristic morphology that occurs predominantly in younger females. Despite presentation with early-stage disease and low nodal burden, these tumors are potentially aggressive, with half of patients developing early distant recurrence.

## 158 Correlation of Clinicopathological Parameters with 21-Gene Traditional Recurrence Scores and TAILORx Among Breast Cancer Patients

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**Disclosures:** Akisha Glasgow: None; Philip Bomeisl: None; Aparna Harbhajanka: None

**Background:** Oncotype DX recurrence score (ODX-RS) is a multi-gene expression-based assay based on expression of 21 tumor-associated genes and can predict risk of invasive breast cancer recurrence and benefit of chemotherapy. Literature is limited on the comparison of traditional ODX with Trial Assigning Individualized Options for Treatment (TAILORx) based on clinicopathological parameters and therapy response. The aim of this study is to examine the difference between traditional ODX and TAILORx score based on clinicopathological parameters and response to therapy.

**Design:** The institutional Breast Cancer Database was queried for patients with newly diagnosed breast cancer (January 2008-June 2018). We analyzed clinical and tumor characteristics including traditional ODX and TAILORx score.

**Results:** There were 674 women in our study cohort. The Caucasian (82.9%) patients made up the majority of the ODX testers compared to African Americans (15.6%) ($p < 0.001$). Overall, 62 (9.2%) vs 113 (16.8%), 214 (31.8%) vs 418 (62%) and 398 (59.1%) vs 143 (21.2%) cases were classified as high risk, intermediate and low risk based on traditional RS cutoffs and TAILORx scores, respectively (Table1). The strongest predictors of a high-risk traditional RS and TAILORx were higher tumor grade and associated higher grade DCIS, invasive ductal carcinoma subtypes (IDC), negative progesterone receptor (PR) and estrogen receptor (ER) status by both immunohistochemistry and oncotype score. The proportion of high-risk traditional RS ranged from 0% (tubular, 0 of 5), 8.3% mucinous (1/12), 0.9 % invasive lobular carcinoma (1/112), 1.4% mixed lobular and ductal (1/70) and 12.4% IDC (58/467) respectively. DFS after chemotherapy was better among higher risk category than lower risk category (Breslow, $p$ <0.001). DFS after radiotherapy and hormonal therapy was better among low and intermediate risk category than high risk category (log rank, $p$ <0.005 and <0.001) (Fig 1, 2).

| Clinicopathological parameters | Low Risk (<18) | Intermediate Risk (18-30) | High Risk(>30) | Total | P value |
|---|---|---|---|---|---|
| **Age((yr, mean ± SD)** | 61.4±10.44 | 58.6±10.40 | 60.0±10.23 | - | 0.223<br>Low/Int: 0.002<br>Int/High: 0.380<br>Low/High: 0.306 |
| **Size** | 1.86±1.17 | 1.93±1.15 | 1.86±0.78 | | Low/Int: 0.412<br>Int/High: 0.628<br>Low/High: 0.998 |
| **ER IHC** | 94.56±4.84 | 92.81±8.22 | 75.67±30.2 | | Low/Int: 0.054<br>Int/High: <0.001<br>Low/High: <0.001 |
| **PR IHC** | 80.0469.96 | 54.08±37.23 | 27.64±35.69 | | Low/Int: <0.001 |

| | | | | | |
|---|---|---|---|---|---|
| | | | | | Int/High: 0.003<br><br>Low/High: <0.001 |
| **ONCO ER** | 10.27±0.94 | 9.65±1.39 | 8.47±1.99 | | Low/Int: 0.001<br><br>Int/High: 0.286<br><br>Low/High: <0.001 |
| **ONCO PR** | 7.86±1.51 | 6.85±1.43 | 5.49±1.48 | | Low/Int: <0.001<br><br>Int/High: <0.001<br><br>Low/High: <0.001 |
| **ONCO HER2** | 9.1±0.57 | 8.92±0.67 | 8.96±1.28 | | Low/Int: 0.073<br><br>Int/High: 0.776<br><br>Low/High: 0.401 |
| **Race** | | | | | 0.882 |
| **White** | 328 (59.2%) | 176 (31.8%) | 50 (9.0%) | 554 (82.9%) | |
| **African Americans** | 61 (58.7%) | 32 (30.8%) | 11 (10.6%) | 104 (15.6%) | |
| **Others** | 3 (50%) | 3 (50%) | 0 (0%) | 6 (0.6%) | |
| **Grade** | | | | | <0.001 |
| **1** | 97 (72.9%) | 34(25.6%) | 2(1.5%) | 133(19.9%) | |
| **2** | 268(63.8%) | 130(31.0%) | 22(5.2%) | 420(63.0%) | |
| **3** | 29(25.4%) | 48(42.1%) | 37(32.5%) | 114(17.1%) | |
| **Total** | 394(59.1%) | 212(31.8%) | 61(9.1%) | 667 | |
| **Subtypes** | | | | | 0.01 |
| **IDC** | 261 (55.9%) | 148(31.7%) | 58(12.4%) | 467(69.4%) | |
| **ILC** | 72(64.3%) | 39(34.8%) | 1(0.9%) | 112(16.6%) | |
| **IMC** | 48(68.6%) | 21(30.0%) | 1(1.4%) | 70(10.4%) | |
| **IMuC** | 8(66.7%) | 3(25%) | 1(8.3%) | 12(1.8%) | |
| **ITC** | 3(60%) | 2(40%) | 0(0%) | 5 (0.7%) | |
| **DCIS** | 5 (71.4%) | 1(14.3%) | 1(14.3%) | 7 (1%) | |
| **Assoc DCIS** | | | | | <0.001 |
| **0** | 140 (35.6%) | 69(32.5%) | 11(17.7%) | 220(33%) | |
| **1** | 28(7.1%) | 7(3.3%) | 2(3.2%) | 37(5.5%) | |
| **2** | 182(46.3%) | 80(37.7%) | 18(29%) | 280(42%) | |
| **3** | 43(10.9%) | 55(25.9%) | 31(50%) | 129(19.3%) | |
| **Not reported** | 0 (0%) | 1(0.5%) | 0(0%) | 1(0.1%) | |
| **Total** | 393(58.9%) | 212(31.8%) | 62(9.3%) | 667 | |
| **N stage** | | | | | 0.173 |
| **0 and (i+)** | 324 (57.2%) | 185(32.7%) | 57(10.1%) | 566(86.1%) | |
| **1/mi** | 61(67.8%) | 26(28.9%) | 3(3.3%) | 90(13.7%) | |
| **2** | 1(0.3%) | 0(0%) | 0(0%) | 1(0.2%) | |
| **Total** | 386 (58.8%) | 211(32.1%) | 60(9.1%) | 657 | |
| **T stage** | | | | | |
| **T1** | 27 (59.7%) | 143 (31%) | 43(9.3 %) | 462(68.6%) | 0.713 |
| **T2** | 113(56.5%) | 68(34%) | 19(9.5%) | 20 (29.7 %) | |
| **T3** | 8(72.7%) | 3(27.3%) | 0 (0%) | 11(1.6%) | |
| **Total** | 397 | 214 | 62 | 673 | |
| **Chemo** | | | | | <0.001 |
| **Yes** | 359 (91.6%) | 129(60.8%) | 12(20.3%) | 500(75.4%) | |
| **No** | 32(8.2%) | 82(38.7%) | 47(79.7%) | 161(24.3%) | |
| **Not known** | 1(0.3%) | 1(0.5%) | 0 (0%) | 2 (0.3%) | |

| | | | | | |
|---|---|---|---|---|---|
| **Total** | 392 (59.1%) | 211 (32%) | 59(8.9%) | 663 | |
| **Radiation** | | | | | 0.834 |
| **Not received** | 137 (35%) | 72(34.3%) | 18(31%) | 225(34.4%) | |
| **Received** | 254(65%) | 138(65.7%) | 40(69%) | 432(65.6%) | |
| **Total** | 391 (59.3%) | 210 (31.9%) | 58 (8.8%) | 659 | |
| **Recurrence** | | | | | 0.610 |
| **Absent** | 378(96.4%) | 203(94.9%) | 55(94.8%) | 636(95.8%) | |
| **Present** | 14(3.6%) | 11(5.1%) | 3(5.2%) | 28(4.2%) | |
| **Total** | 392(59%) | 214(32.2%) | 58(8.7%) | 664 | |
| **Mets** | | | | | 0.246 |
| **Yes** | 377(97.2%) | 205(96.7%) | 52(92.9%) | 634(96.6%) | |
| **No** | 11(2.8%) | 7(3.3%) | 4(7.1%) | 22(3.4%) | |
| **Total** | 388 | 212 | 56 | 656 | |
| **Recurrence and Mets** | | | | | 0.03 |
| **Absent** | 372(94.9%) | 196 (91.6%) | 50 (86.2%) | 618(93.1%) | |
| **Present** | 20(5.1%) | 18(8.4%) | 8(13.8%) | 46(6.9%) | |
| **Total** | 392 (59%) | 214 (32.2%) | 58(8.7%) | 664 | |
| **TAILORx** | | | | | <0.001 |
| | | | | | |
| **Low risk (<11)** | 143(35.9%) | 0 | 0 | 143(21.2%) | |
| **Intermediate(11-25)** | 25 (64.1%) | 163 (76.2%) | 0 | 418(62%) | |
| **High risk (>25)** | 0 | 51(23.8%) | 62 (100%) | 113(16.8%) | |
| **Total** | 398(59.1%) | 214 (31.8%) | 62 (9.2%) | 674 | |

Figure 1 – 158

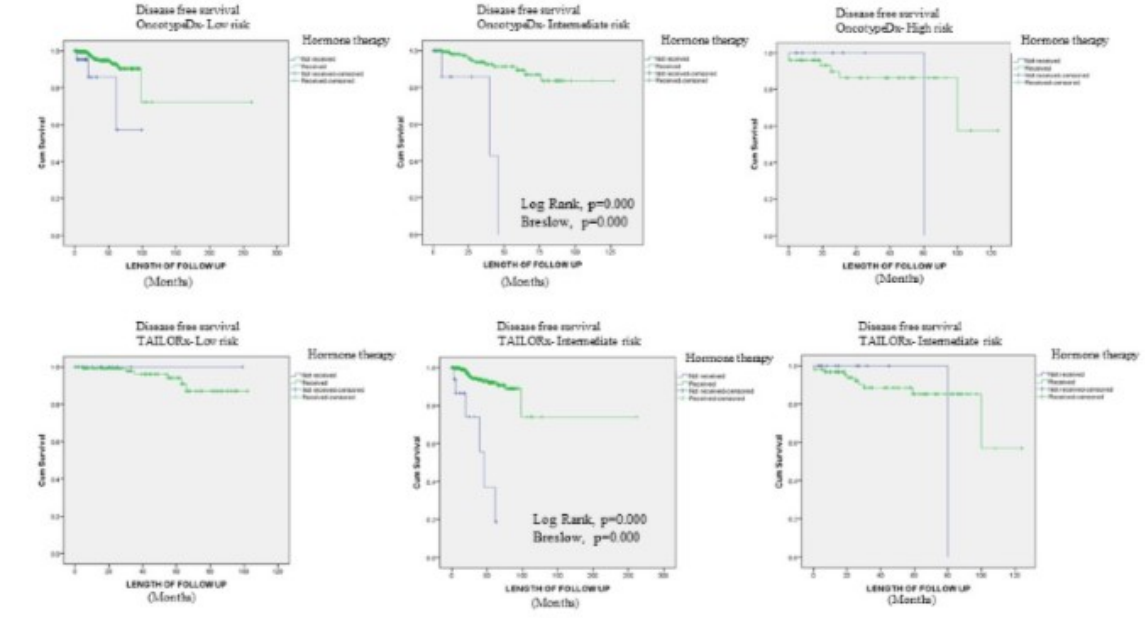
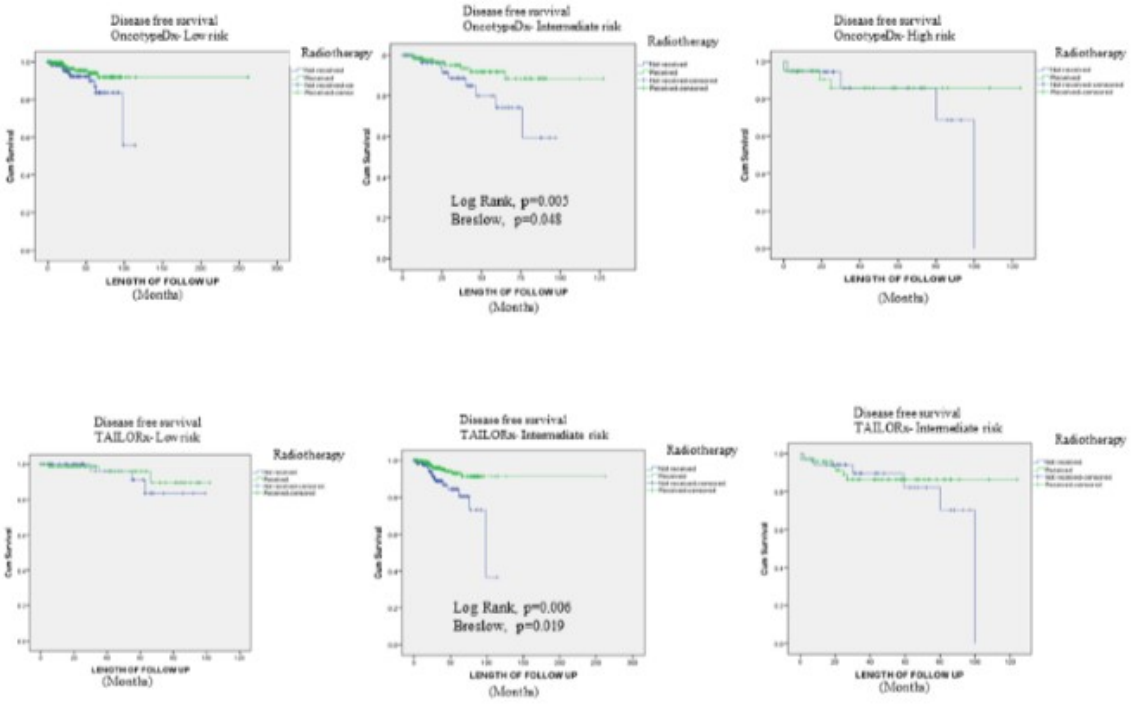


Figure 2 – 158




**Conclusions:** The number of cases decreased approximately to half in low risk category and doubled in intermediate and high-risk category of TAILORx compared to traditional RS with no change in predictors of a high-risk category based on clinicopathological parameters and therapy response.

## 159 HER2 positive breast cancers with HER2:CEP17 ratio ≥2 and copy number >4 and <8 should require additional workup as recommended for Press groups G2-G4 in the 2018 ASCO/CAP HER2 update

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**Disclosures:** Paula Gonzalez Ericsson: None; Brent Rexer: None; Ferrin Wheeler: None; Ashwini Yenamandra: None; Melinda Sanders: None

**Background:** Breast cancers tested for HER2 amplification (AMP) by fluorescence in situ hybridization (FISH) according to 2013 guidelines can be divided in to 5 groups (G1-G5; *Press. APLM 2016*) based HER2 copy number (CN) and HER2:CEN17 ratio. Only G1 (HER2 AMP) and G5 (HER2 not AMP) demonstrate consistent over-expression (3+) vs. low/lack of expression (1+/0) by immunohistochemistry (IHC) and significant correlation with response to HER2-targeted therapy (*Press JCO 2016)*. Based on uncertain/lack of benefit in clinical trials, the 2018 ASCO/CAP HER2 Guideline Update now requires performance of HER2 IHC and scoring of additional cells for G2-G4. However, among tumors within G1, HER2:CEP17 ratio and especially HER2 CN can vary widely. Approximately 22% of G1 tumors score as IHC 0/1+ (*Press APLM 2016).* Furthermore, 88% of tumors with low level AMP (HER2:CEP17 ratio ≧ 2.0 and HER2 CN≧4.0 but <6) score as IHC 0/1(*Press JCO 2016),* suggesting lack of clinically significant AMP. Guidance with reporting of such cases is not addressed in the 2018 Update. Refined criteria to identify G1 patients with clinically insignificant low-level HER2 amplification are needed.

**Design:** Patients who received neoadjuvant anti-HER2 +/-chemotherapy (NAT) at our institution were retrospectively classified as G1-G4 based on results of HER2 FISH testing per 2013 guidelines. We examined the relationship between pathologic response (pCR), HER2 CN, HER2:CEN17 ratio and HER2 IHC among G1 patients.

**Results:** 100 consecutive patients receiving NAT were subclassified as: G1=93%, G2=2%, G3=2% and G4=3%. Restricting analysis to G1, 37% achieved pCR, and 65% near pCR/pCR (RCB 0-I). 77% of patients had HER2 CN≥8 (ave 22.7). 43% with HER2 CN≥8 achieved pCR, compared to 10% of tumors with CN<8 (OR=6.8, p=0.0075). pCR was significantly associated higher mean HER2 CN (range 4.2-

44.6; ave 24 vs 16, p=0.0006), HER2 IHC (n=39, p=0.002 as a continuous variable; IHC 3+ p=0.075), and HER2:CEP17 ratio (range 2-18.8, ave 8.7 vs 6.5, p=0.038; ratio≥5 OR=2.84 p=0.03), but not with ER status (p=0.8327).

**Conclusions:** HER2:CEP17 ratio≥5 and CN≥8, as well as IHC3+, associate with pCR in G1, serving as a surrogate of long term outcome. Tumors showing CN<8 were significantly less likely to benefit from HER2 targeted NAT. This population represents approximately 7% of all BC (*Press JCO 2016*), 85% of which were 0/1+IHC. We propose tumors with HER2:CEP17 ratio ≥2 and CN≥4 but <8 should require additional workup, as recommended for G2-G4 in the 2018 HER2 update.

## 160 Flat Epithelial Atypia (FEA) in Breast Core Needle Biopsy (CNB): Is Excision Necessary?

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**Disclosures:** Anne Grabenstetter: None; Elena Salagean: None; Edi Brogi: None

**Background:** FEA consists of distended acini lined by 2-5 layers of ductal cells with low grade atypia without architectural complexity. No consensus exists on whether FEA requires follow-up excision (EXC). This study evaluated the upgrade rate of FEA.

**Design:** We retrospectively identified all consecutive CNBs with FEA and no other lesion mandating EXC obtained between 1/2013-7/2018. Clinical and imaging features were recorded. We reviewed all CNB slides and noted the number and size of FEA foci. An upgrade was defined as IC or DCIS in the EXC. The EXC slides of upgraded cases were reviewed.

**Results:** Out of ~15,700 in-house CNBs in the study period, there were 97 CNBs with FEA from 97 patients. We excluded CNBs from 36 patients with prior/concurrent ipsilateral/ contralateral invasive carcinoma (IC) or ductal carcinoma in situ (DCIS), 5 CNBs with concurrent atypical ductal hyperplasia (ADH), and 10 CNBs without EXC. After re-review of the CNB slides we further excluded 8 that were reclassified: 2 flat lesions with marked nuclear atypia, 5 focal ADH (= ADH <2 mm, 1 focus), 1 benign without atypia. On EXC, the 2 CNBs with marked atypia yielded DCIS; the 5 CNBs with ADH yielded: 1 IC (1.2 cm grade II/III), 1 DCIS (2 mm focus), and 3 ADH; the CNB reclassified as benign yielded ADH. The final FEA study cohort consisted of 38 CNBs from 38 patients (median age 50 years, range 35-73). The CNB targeted mammographic calcifications in 34 (89%) cases, MRI non-mass enhancement in 3 (8%), and 1 (3%) sonographic mass. FEA was present alone in 33 (87%) cases and with classic lobular neoplasia (LN) in 5 (13%). There were 2 (5%) upgrades to IC at EXC (Table 1) and FEA was the only lesion in the CNBs. In both cases the biopsy site was identified in the EXC in a tissue section without IC. There was no upgrade in 36 (95%) cases (8 FEA, 4 LN without FEA, and 24 benign breast). The median number of FEA foci and the median size of the largest FEA focus in the CNBs with or without upgrade was comparable.

Table 1: Clinicopathologic Features of Upgraded Cases

| Case | Age | Mammographic Calcifications | CNB Findings | EXC Findings |
|---|---|---|---|---|
| 1 | 73 | 10 mm pleomorphic | 2 FEA foci, largest 3 mm | IDC, grade II/III, 2.2 mm |
| 2 | 46 | Suspicious | 1 FEA focus, 3.7 mm | Tubular carcinoma, grade I/III, two foci (2.0 mm and 1.0 mm) |

Note: IDC - invasive ductal carcinoma

**Conclusions:** In this data set, the upgrade rate of FEA was 5%. All (2) upgrades consisted of minute, incidental low-grade ICs not associated with the biopsy site. Further evaluation of radiologic-pathologic correlation is pending. Our findings document intrinsic difficulties in the diagnosis of FEA. However, when strict diagnostic criteria are applied, the upgrade rate is low and non-operative management of FEA can be considered in patients without prior/concurrent carcinoma and no other lesion mandating excision.

## 161 Comparison of the Clinical and Pathological Features of The Different Grades of Phyllodes Tumours of the Breast and distinguishing them Preoperatively via a Nomogram

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*[1]Singapore, Singapore, [2]KK Hospital, Singapore, Singapore, [3]KK Women's and Children's Hospital, Singapore, Singapore, [4]Duke-Nus Medical School Singapore, Singapore, Singapore*

**Disclosures:** Mihir Gudi: None; May Ying Leong: None; Zhiyan Yan: None; Fazliana Abd Rashid: None; Sorsiah Mansor: None; Swee Ho Lim: None; Sze Yiun Teo: None; Saffari Seyed Ehsan: None

**Background:** Phyllodes tumours are rare tumours of the breast accounting for less than 1% of all breast tumours. They are distinguished histologically into benign, borderline and malignant subtypes. Our study aims to compare the clinical and pathological characteristics between the subtypes of phyllodes tumours of the breast with the purpose of distinguishing them pre operatively.

**Design:** This is a single institution retrospective study of all excision proven phyllodes tumours of the breast. Patients without both imaging and histological findings were excluded. Imaging and histological features were reviewed by radiologists and pathologists to ensure completion of database. Multivariable logistic regression analysis was used to investigate the association between the clinical parameters and clinical outcome. A nomogram was produced based on preoperative features including age, fat within the lesion, increased mitosis and sonographic size> 30mm.

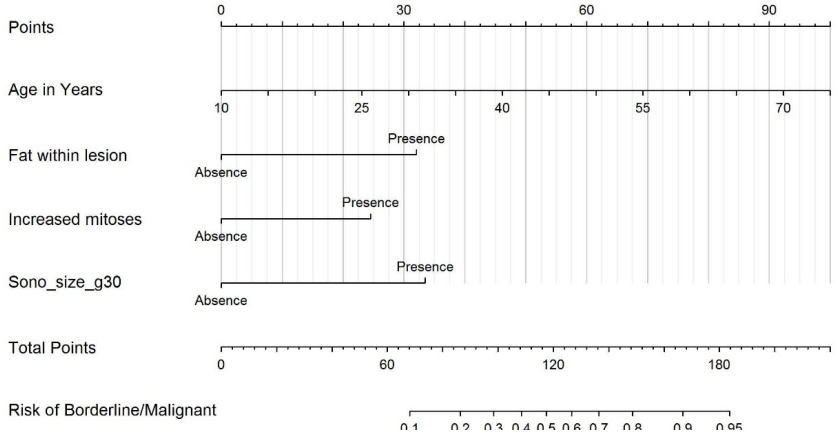
**Results:** A total of 302 patients met inclusion criteria for the study. Multivariable analysis indicated that age (OR 1.09 (1.05, 1.13) p <0.0001), cores with stroma only (OR 3.08 (1.17, 8.11) p=0.0225), fat within the lesion OR 5.09 (2.21, 11.8) p<0.0001, fragmentation of cores (OR 0.37 (0.14, 0.98) p=0.0452, increased mitosis (OR 2.46 (1.06.5.69) p=0.0359), sonographic size> 30mm (OR 2.79 (1.29, 6.03) p=0.009), sonographic shape (OR 0.31 (0.15, 0.66) p=0.0022) were significant in distinguishing between benign and borderline/malignant phyllodes tumours of the breast. The generated nomogram is 85% accurate in predicting the presence of borderline/ malignant phyllodes tumours of the breast ( Fig 1).

Fig1 : Nomogram predicting the likelihood of borderline/malignant phyllodes tumours of the breast

| Variable | Adjusted Odds Ratio* (95% CI) | P Value |
|---|---|---|
| Age | 1.09 (1.05, 1,13) | <0.001 |
| Cores with Stroma Only | 3.08 (1.17, 8.11) | 0.0225 |
| Fat within the Lesion | 5.09 (2.21, 11.8) | 0.0001 |
| Fragmentation of Cores | 0.37 (0.14, 0.98) | 0.0452 |
| Increased Mitosis | 2.46 (1.06, 5.69) | 0.0359 |
| Sonographic Size >30mm | 2.79 (1.29, 6.03) | 0.0090 |
| Sonographic Shape (Irregular vs Oval) | 3.23 (1.52, 6.67) | 0.0022 |
| * *Variables were chosen via stepwise selection approach* | | |

Table 1 Multivariable analysis of histological and imaging parameters

Figure 1 - 161



**Conclusions:** Phyllodes tumour of the breast are rare fibroepithelial lesions of the breast and there is emerging data that the benign subtype can be treated less aggressively with a smaller margin. Our study has identified 4 pre-operative features that can be entered into nomogram that can be used in an outpatient setting. A clinician can utilize this in surgical planning and patient counselling.

## 162 Pseudoangiomatous stromal hyperplasia of Breast encountered in MRI-guided core needle biopsy of breast: A clinical, radiologic and pathologic study of 88 subjects

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**Disclosures:** Ameer Hamza: None; Elsa Arribas: None; Gary Whitman: None; Constance Albarracin: None; Alejandro Contreras: None; Roland Bassett: None; Savitri Krishnamurthy: None

**Background:** Pseudoangiomatous stromal hyperplasia (PASH) of the breast is commonly detected on magnetic resonance imaging (MRI) as areas of mass-like or non-mass like enhancements that are subject to core needle biopsy (CNB). Radiologic-pathologic correlation of this benign entity has significant implications for the clinical management of the patients. The primary objective of our study was to evaluate the clinical, radiologic and pathologic features of PASH encountered in MRI-guided CNBs.

**Design:** This is a retrospective study of PASH diagnosed by MRI-guided CNBs in our institution from 2008 to 2015. We recorded pertinent details including patient age, race, history of hormonal therapy, oral contraceptive (OCP) use, MRI appearance: mass-like or non mass-like, size of the lesion; pathologic findings on CNB: number of cores showing PASH, maximum dimension, cellularity as defined by increased numbers of spindle cells in the slit-like spaces, presence of ductal epithelial hyperplasia and calcifications. Wilcoxon rank-sum tests and Fisher's exact test were used to compare the variables between mass-like and non-mass like lesions on MRI.

**Results:** We evaluated 88 women ranging in age from 28 to 74 years with CNB diagnosis of PASH with underlying mass-like and non-mass like MRI findings in 37 (42%) and 51 (58%) patients. 69% patients were Caucasians, 6% African American and 25% others. A history of OCP use was available in 79 (90%) and hormonal therapy in 88 (100%). The radiologic size of mass-like lesion ranged from 0 to 3 cm (mean 0.91 cm) and non mass-like from 1 to 9 cm (mean 2.57 cm). The number of CNBs ranged from 6 to 46 (mean 13) and 1 to 22 (mean 5.8) cores showed PASH ranging in size from 0.1 to 1.4 cm on an individual core. The areas of PASH were associated with ductal epithelial hyperplasia in 3% cases and with calcifications in 3.6% cases. Mean radiologic size of non-mass like enhanced PASH was greater as compared to mass like enhanced PASH (2.6 ± 1.9 cm versus 0.9 ± 0.8 cm, $p = < 0.0001$). There was no significant correlation of hormone therapy or OCP use among these two groups ($p = 0.33$ and 0.50 respectively).

**Conclusions:** PASH lesions present more often as non-mass like enhancement and they tend to be larger in size in comparison to those with mass-like enhancement on MRI of the breast. A history of oral contraceptive use and hormonal therapy was commonly associated with PASH. Increased cellularity, ductal hyperplasia and calcifications were infrequently associated with PASH.

## 163 Genomic Profiling of Morphologic Variants of Lobular Carcinoma In Situ (LCIS)

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**Disclosures:** Beth Harrison: None; Faina Nakhlis: None; Deborah Dillon: None; Stuart Schnitt: None; Tari King: None

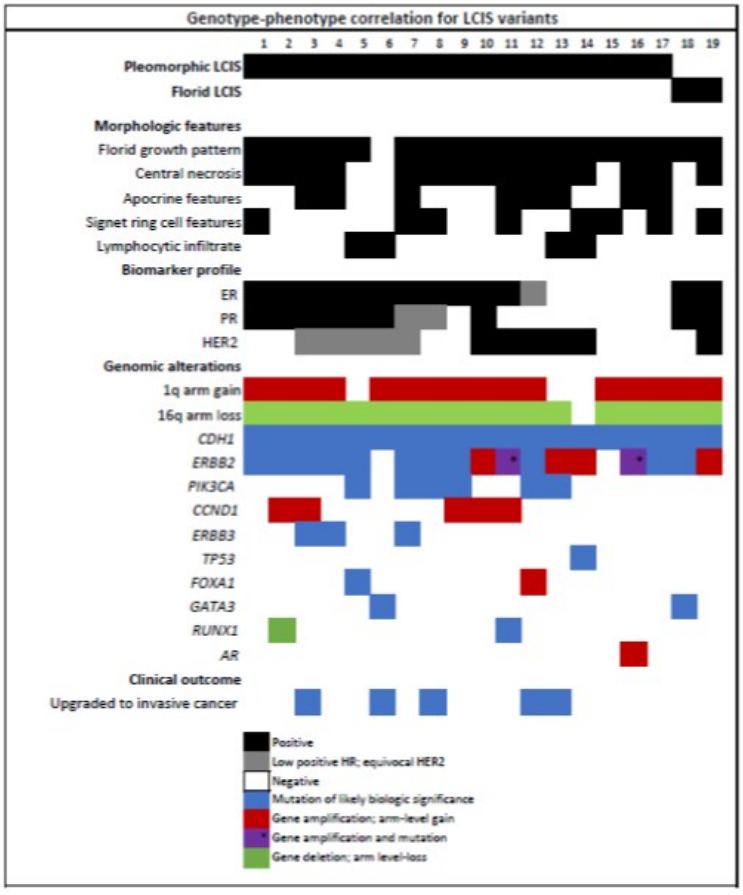
**Background:** Pleomorphic LCIS (PLCIS) and florid LCIS (FLCIS) are morphologic variants distinguished from classical LCIS by marked nuclear pleomorphism and/or an expansile growth pattern with necrosis. PLCIS was historically confused with ductal carcinoma in situ (DCIS) and is still often managed like DCIS with complete excision +/- radiation due to uncertainties regarding the subsequent breast cancer risk associated with these lesions. Given the rarity of LCIS variants, little data exist regarding their molecular pathogenesis, natural history and optimal management. The purpose of this study is to genomically profile LCIS variants to gain further insight into their biology and their similarity to classical LCIS.

**Design:** Nineteen cases of pure LCIS variants (17 PLCIS, 2 FLCIS) diagnosed on core biopsy at our institution from 2006-2017 were included, 5 of which were upgraded to invasive cancer at excision. Macrodissected lesions were analyzed by a hybrid-capture next generation sequencing assay that interrogates the full coding sequences of 447 genes for mutations and copy number variations (CNVs).

**Results:** LCIS variants, all confirmed as E-cadherin negative, represented a variety of pathologic phenotypes. Most cases of PLCIS contained a florid growth pattern (18), a subset with apocrine (8) and/or signet ring cell (8) features or a dense lymphocytic infiltrate (4). Biomarker profiles included HR+/HER2- (9), HR+/HER2+ (3), HR-/HER2+ (2) and HR-/HER2- (3). FLCIS cases contained a florid growth pattern, central or single cell necrosis and HR+/HER2- and HR+/HER2+ profiles. All LCIS variants had alterations consistent with a lobular phenotype including 1q gain (16), 16q loss (18) and CDH1 mutation (19). Highly recurrent *ERBB2* alterations were noted including mutations (13) and amplifications (6). Other significant alterations included mutations in *PIK3CA* (6), *ERBB3* (3), *GATA3* (2), *FOXA1* (1)

and *RUNX1* (1) and amplifications of *CCND1* (5) and *AR* (1). A *TP53* mutation was identified in 1 case of HR-/HER2+ PLCIS with signet ring cell features that lacked 1q gain and 16q loss. No differences were noted between cases that did or did not upgrade.

Figure 1 - 163




**Conclusions:** PLCIS and FLCIS contain genetic alterations characteristic of lobular neoplasia; however, these LCIS variants are distinguished from classical LCIS reported in the literature by highly recurrent *ERBB2* alterations. A single case with *TP53* mutation and no 1q gain and 16q loss raises the possibility of a subset of lesions that have a distinct pathogenesis.

## 164 Pathologic Evaluation of Gender-Affirming Surgical Specimens in Female-to-Male Transitioning Individuals

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**Disclosures:** Andrea Hernandez: None; Christopher Schwartz: None; Ugur Ozerdem: None; Kristen Thomas: None; Rachel Bluebond-Langner: None; Farbod Darvishian: None

**Background:** Gender dysphoria (GD) is the distress caused by the incongruence in the sex assigned at birth and the individual's gender. Bilateral mastectomy or chest masculinization is one of the treatments for GD in transmasculine individuals. At our institution, we typically submit 16 blocks per mastectomy specimen (4 per quadrant) akin to prophylactic mastectomy guidelines. Our study aimed to optimize gross handling protocols and assess histopathologic findings in the mastectomy specimens of individuals with GD.

**Design:** We identified all mastectomies for individuals with GD from January 2015 to August 2018 and sequentially retrieved the clinicopathologic information by interrogating the pathology archives. We used reduction mammoplasty (RM) cases for macromastia from the same time period as control. We recorded age, diagnosis and the number of slides examined per case. Significant pathologic findings were defined as atypical lobular hyperplasia (ALH), atypical ductal hyperplasia (ADH), lobular carcinoma in situ (LCIS), ductal carcinoma in situ (DCIS) or invasive carcinoma. Statistical analyses were performed using GraphPad Prism version 7.00.

**Results:** See table for clinicopathologic findings. All individuals with GD (n=211) underwent bilateral mastectomy. Significant pathologic findings were present in 6 of 211 (2.8%) cases as follows: ADH (n=5) and LCIS (n=1). By comparison, 19 of 273 (7%) RM specimens for macromastia yielded significant pathologic findings as follows: ALH (n=11), ADH (n=4), LCIS (n=2), DCIS (n=1) and invasive lobular carcinoma (n=1). The mean age of individuals with GD who had significant pathologic findings was 32.5 years (range 22 to 55) compared to the overall mean age of 28.1 years in this group.

**Table.** Clinicopathologic Features of Mastectomies in Individuals with Gender Dysphoria (GD) and Reduction Mammoplasties (RM) in Macromastia

| | Mean Age (y) | Total Number of Slides Examined | Average Number of Slides Examined/Case | Number of Cases with Significant Findings (%) | Incidence of Cases with Significant Findings/Slide (%) |
|---|---|---|---|---|---|
| Mastectomy for GD (n=211) | 28.1 | 6,225 | 29.5 | 6 (2.8%) | 0.096% |
| RM for Macromastia (n=273) | 40.6 | 2,930 | 10.7 | 19 (7%) | 0.65% |
| p-value | <0.0001 | <0.0001 | <0.0001 | 0.04 | 0.03 |

**Conclusions:** Our study demonstrates that we handle mastectomy specimens in individuals with GD by examining 2.5 times more slides compared to RMs, with a 2.8 times lower yield of significant pathologic findings. While the difference between the incidences of significant pathologic findings may be attributable to factors like age, hormonal treatment (androgenic vs estrogenic), genetic predisposition and family history, it does not justify the excessive tissue submission in mastectomies in individuals with GD. Pathologists can safely examine mastectomy specimens in individuals with GD more conservatively than our current guidelines without compromising the optimal care of individuals undergoing chest masculinization.

## 165 Secretory Carcinoma of the Breast: Clinicopathologic Profile of 14 Cases Emphasizing Distant Metastatic Potential

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**Disclosures:** Raza Hoda: None; Edi Brogi: None; Fresia Pareja: None; Gouri Nanjangud: None; Melissa Murray: None; Britta Weigelt: None; Jorge Reis-Filho: *Advisory Board Member*, Volition Rx; *Advisory Board Member*, Paige.AI; *Consultant*, Goldman Sachs; Hannah Wen: None

**Background:** Secretory carcinoma of the breast (SCB) is a rare histologic type of breast carcinoma with a generally indolent clinical behavior. However, standardized treatment approaches for SCB have not been established, given the rarity of the disease, and studies on particularly aggressive SCB cases remain limited.

**Design:** The surgical pathology and consultation service records of a single large, academic cancer center were electronically searched for the diagnosis of SCB from 1992 to 2017. Clinical information, pathologic diagnoses, results of molecular testing, including next-generation sequencing and fluorescent *in situ* hybridization, and treatment and outcome data, if available, were reviewed.

**Results:** 14 patients with a diagnosis of SCB were identified, including 12 women and 2 men. Median age of patients was 56 years (mean,48 years;range,8-81 years). Patients presented with either palpable (8 cases) or radiographic (4 cases) abnormalities. All cases were unilateral (left:8, right:6). Surgical procedures included excisional biopsies (10 cases) and ipsilateral mastectomies (4 cases). 1 case was diagnosed as *in situ* SCB arising in a complex papilloma. In 10 cases, estrogen receptor (ER), progesterone receptor (PR) and HER2 results were obtained, all but 1 were ER/PR negative or had focal positivity in no more than 10% of tumor cells. All cases lacked HER2 overexpression. Molecular testing was performed in 3 cases, all confirmed to harbor the characteristic *ETV6-NTRK3* translocation. Sentinel lymph node biopsy was performed in 10 cases, and 2 patients had axillary lymph node metastasis. Adjuvant therapies incorporated chemotherapy (6 cases), hormonal therapy (5 cases) and radiation therapy (4 cases). Clinical follow-up ranged from 21 to 212 months (median, 70 months; mean, 89 months). 2 patients succumbed to malignancies other than SCB, and 10 patients had no evidence of disease after initial treatment. 2 patients, however, developed distant metastasis of SCB (Figures 1 & 2). Both cases with distant metastasis were confirmed as SCB by molecular testing. One of the patients with distant metastases received treatment with a pan-Trk inhibitor and showed dramatic clinical response.

**Table 1.** Clinical and pathologic profile of 14 cases of secretory carcinoma of the breast.

| Case no. | Sex (F/M) | Age at Dx (y) | Presentation | Laterality | Tumor Size (cm) | ER* | PR* | HER2* | *ETV6-NTR3* Test Result† | Locoregional Treatment | Systemic Treatment | Axillary Nodes | Follow-up, months |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| #1 | F | 8 | Palp mass | Left | NA | Neg | Neg | Neg | Pos | BCS‡ | AC | NA | Alive with metastatic SCB, 88 |
| #2 | M | 26 | Palp mass | Left | 2.0 | >=10 % | <10 % | Neg | Pos | SM | None | Neg | Alive with metastatic SCB, 59 |
| #3 | F | 11 | Palp mass | Right | NA | <10% | Neg | Neg | ND | BCS | None | Neg | NED, 156 |
| #4 | M | 19 | Palp mass | Right | 2.5 | NA | NA | NA | ND | SM | AC | Pos | NED, 29 |
| #5 | F | 31 | Palp mass | Left | 1.8 | Neg | Neg | Neg | ND | SM | AC | Neg | DOC, 56 |
| #6 | F | 46 | Palp mass | Left | 1.4 | <10% | <10 % | Neg | ND | BCS + RT | AC + HT | Neg | NED, 80 |
| #7 | F | 54 | NA | Right | 1.0 | NA | NA | NA | ND | BCS | None | NA | NED, 153 |
| #8 | F | 57 | Mammo | Left | 0.8 | <10% | <10 % | Neg | ND | BCS + RT | AC + HT | Neg | NED, 33 |
| #9 | F | 59 | Mammo | Right | *In situ* only | NA | NA | NA | ND | BCS | None | NA | NED, 212 |
| #10 | F | 63 | Mammo | Right | 1.0 | <10% | Neg | Neg | ND | BCS + RT | HT | Neg | NED, 54 |
| #11 | F | 67 | Mammo | Left | 0.9 | Neg | Neg | Neg | ND | BCS + RT | AC + HT§ | Neg | NED, 121 |
| #12 | F | 69 | Palp mass | Left | 2.1 | >=10 % | Neg | Neg | ND | SM | AC‖ + HT | Neg | DOC, 21 |
| #13 | F | 75 | NA | Right | NA | Neg | Neg | Neg | Pos | BCS‡ | NA | Pos | NA |
| #14 | F | 81 | Palp mass | Left | 4.5 | NA | NA | NA | ND | BCS‡ | NA | NA | NA |

Abbreviations: AC, adjuvant chemotherapy; BCS, breast-conserving surgery; DOC, died of other causes; Dx, diagnosis; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; F, female; HT, hormonal therapy; M, male; Mammo, screening mammogram; NA, information not available; ND, not done; NED, no evidence of disease; Neg, negative; Palp, Palpable; Pos, positive; PR, progesterone receptor; RT, radiation therapy; SCB, secretory carcinoma of breast; SM, simple mastectomy; y, years; >=, greater than or equal to.

* Percentage of stained nuclei of tumor cells.

† Testing included next-generation sequencing and fluorescent *in situ* hybridization.

‡ Data regarding if radiation therapy was utilized is not available for 3 patients.

§ Core biopsy at an outside institution showed 20% of tumor cells positive for ER.

‖ This patient underwent adjuvant chemotherapy for a separate malignancy.

Figure 1 – 165

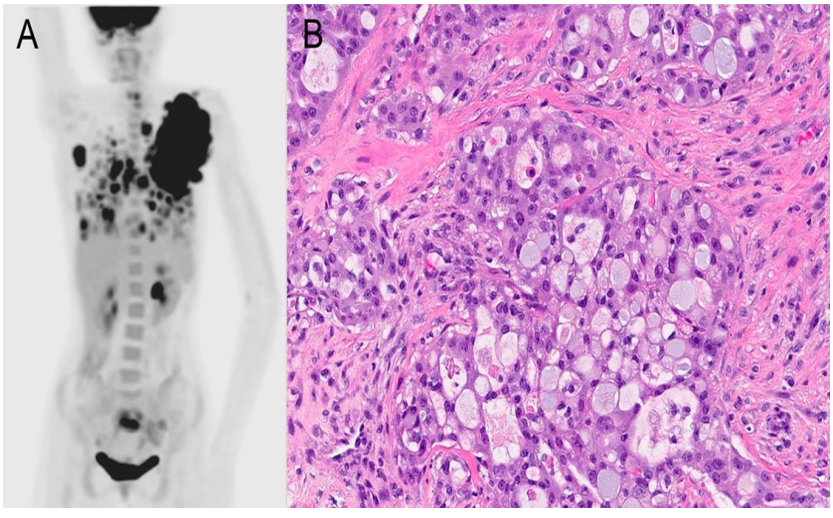
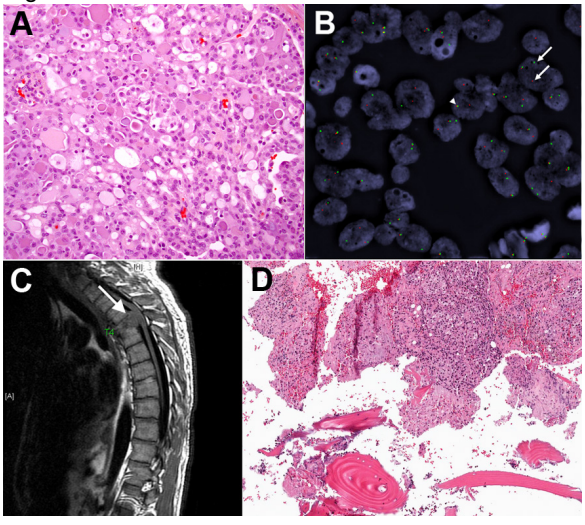



Figure 2 – 165



**Conclusions:** Although SCB is generally associated with a favorable prognosis, our study demonstrates that a subset of SCB may develop distant metastases. Further studies are warranted to identify markers predictive of more aggressive clinical behavior in this rare breast cancer subtype.

## 166 Immunohistochemical comparison of metaplastic breast carcinoma with triple negative breast carcinoma lacking metaplastic features in relation to checkpoint immune biomarkers

Yanjun Hou[1], Hiro Nitta[2], Peter Banks[3], Anil Parwani[4], Zaibo Li[5]
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**Disclosures:** Yanjun Hou: None; Hiro Nitta: *Employee*, Roche Diagnostics; Peter Banks: *Consultant*, Ventana Medical Systems, Inc.; Anil Parwani: None; Zaibo Li: None

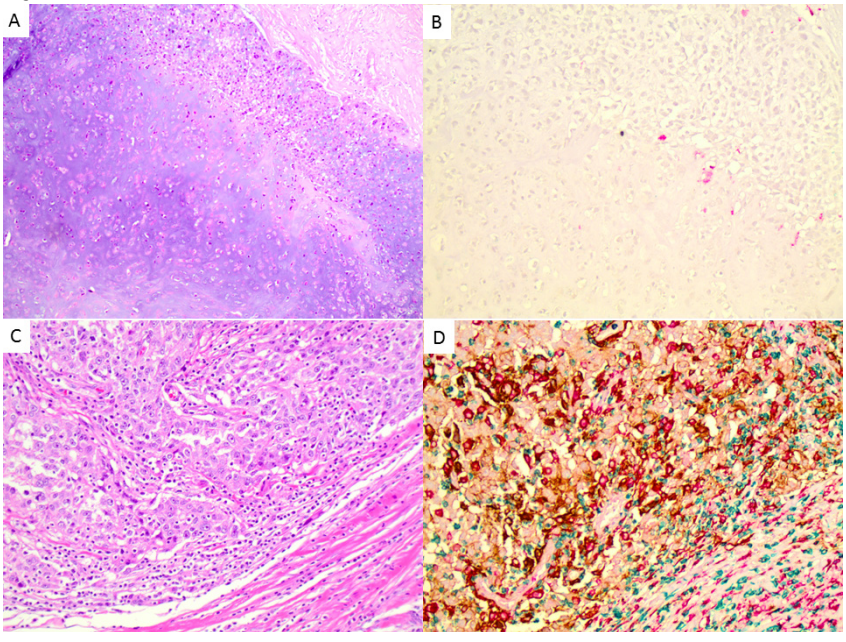
**Background:** Metaplastic breast carcinoma (MBC) is a rare type of aggressive disease, predominantly falling within the hormone/Her2 grouping of triple negative breast carcinoma (TNBC). The prognosis of MBC is worse than that of TNBC in general. Our purpose was to compare the checkpoint immune marker expression in MBC as compared to that in non-metaplastic TNBC.

**Design:** 44 MBCs and 119 TNBCs were initially included in the study. Average age for BMC was 55.4 years. Checkpoint immune markers, including PD-L1, CD8 and CD163, were assessed by multiplex immunohistochemistry (IHC) in 27 MBCs (with sufficient remaining tissue) and all 119 TNBCs (Figure 1: Images of two cases of MBC represent contrasting patterns of immune reaction and PD-L1 expression, as detected with multiplex immunohistochemistry (anti-CD8 in green, anti-CD163 in red, and anti-PD-L1 in brown. A, B: Invasive metaplastic carcinoma with no PD-L1 expression, only scattered CD163+ cells and very rare CD8+ cytotoxic T-cells in peritumoral stroma. C, D: Invasive metaplastic carcinoma with strong PD-L1 expression in tumor cells and stromal cells, diffuse CD163+ cells and CD8+ cytotoxic T-cells).

**Results:** The mean patient age for the MBC group (n=44) was six years older than for the TNBC group (n=119) (p=0.03). MBCs (n=27) demonstrated greater amount of CD163 in the stroma (96.3% vs. 79.8%, p=0.0468) and PD-L1 in the tumor (29.6% vs. 10.1%, p=0.0133) than TNBCs (n=119). However, significantly more TNBCs were positive for CD8 in the tumor than MBCs (44.5% vs. 18.5%, p=0.0158) (Table 1).

| | TNBC (n=119) | BMC (n=27) | Total | P-value |
|---|---|---|---|---|
| Age | 51.9 (11.8) | 57.8 (15.3) | 53.0 (12.7) | 0.0281 |
| Peritumoral-CD8 (?10%) | 94 (79.0%) | 21 (77.8%) | 115 (78.8%) | 1.0000 |
| Intratumroal-CD8 (?10%) | 53 (44.5%) | 5 (18.5%) | 58 (39.7%) | 0.0158 |
| Intratumoral-CD163 (?10%) | 55 (46.2%) | 13 (48.1%) | 68 (46.6%) | 1.0000 |
| Peritumoral-CD163 (?10%) | 95 (79.8%) | 26 (96.3%) | 121 (82.9%) | 0.0468 |
| Tumoral PD-L1 (?1%) | 12 (10.1%) | 8 (29.6%) | 20 (13.7%) | 0.0133 |
| Stromal PD-L1 (?1%) | 87 (73.1%) | 16 (59.3%) | 103 (70.5%) | 0.1665 |
| Overall PD- L1 (?1%) | 87 (73.1%) | 15 (55.6%) | 102 (69.9%) | 0.1025 |

Figure 1 - 166



**Conclusions:** Our findings, that MBCs had more tumoral PD-L1 and stromal CD163 but less intratumoral CD8 expression than non-metaplastic TNBCs, suggest that tumor immune suppression escape is generally present in TNBCs but to a greater extent in MBCs.

## 167 IgA+ Tumor-Infiltrating Plasma Cells Correlate with Tregs and PD-L1 in Triple Negative Breast Cancers

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**Disclosures:** Shaomin Hu: None; Linlin Yang: None; Joseph Albanese: None; Dale Small: None; Murali Janakiram: None; Susan Fineberg: None

**Background:** Various cellular components of tumor-infiltrating lymphocytes (TILs) have been associated with breast cancer prognosis, but the role of IgA+ tumor-infiltrating plasma cell (TIPCs) has rarely been studied. Studies revealed that IgA+ TIPCs can promote prostate cancer progression through inhibiting anti-tumor cytotoxic T-cell (CTL) response, and is associated with melanoma progression. Our own studies indicated IgA+ plasma cells have regulatory function by delivering regulatory T cells (Tregs) to target tissue. Here we hypothesize that IgA+ TIPCs may correlate with Tregs and be potentially associated with worse prognosis of breast cancers.

**Design:** Thirty-nine age- and stage-matched primary breast triple negative invasive ductal carcinoma cases were identified, 20 with metastasis and 19 without metastasis. Sections were stained for IgA, CD8 and Foxp3 (for Treg); positive cells were recorded as cell counts per 10 representative high power fields (HPFs). Corresponding H&E and PD-L1 stained slides were evaluated for stromal TILs (sTILs) and tumor cell PD-L1 expression. Student t test and Fisher's exact test were used for analysis.

**Results:** As expected, there was significant correlation of high sTILs (> 30%) with less metastasis (p=0.02). Consistent with the fact that immune suppressive and immune promoting type inflammatory cells both increase with high TILs, the non-metastasis group had more stromal Tregs (194 ± 162 per 10 HPFs, vs 87 ± 97 in metastasis group, p=0.02) and CD8+ CTLs (798 ± 580 per 10 HPFs, vs 431 ± 354 in metastasis group, p=0.03). Similarly, we found a correlation of high IgA+ TIPCs with high sTILs (p=0.01). However, further analysis revealed that high IgA+ TIPCs positively correlated with high Tregs and PD-L1 expression ($p<0.05$), both of which are well-known immune-suppressors, but not with anti-tumor CD8+ CTLs. When we normalized the absolute IgA+ TIPC count to IgA+ TIPC/CD8+ CTL ratio, it remained correlated with Treg/CD8+ CTL ratio (p=0.02) but not with sTILs. We didn't reveal a significant association of IgA plasma cells with metastasis, either using absolute IgA+ TIPC count or IgA+ TIPC/CD8+ CTL ratio, possibly due to limited case numbers.

**Conclusions:** Our results in this small cohort suggested that IgA+ TIPC count positively correlate with other known immune-suppressors including Tregs and PD-L1, suggesting they may function as tumor-promoting regulator. A large cohort will be further studied to correlate IgA+ TIPC with prognosis of triple negative breast cancer.

## 168 The Spectrum of Pathologic Diagnoses in Non-Sentinel Axillary Lymph Node Biopsy

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**Disclosures:** Wei Huang: None; Xiaoyu Tang: None; Jozef Malysz: None; Zhaohai Yang: None

**Background:** Axillary lymph node is the most common metastatic site for breast carcinoma, and diagnostic lymph node biopsy is a standard procedure for patients with a history of breast carcinoma presenting with abnormally enlarged axillary node. However, the axillary node shows a spectrum of differential diagnoses beyond metastatic breast carcinoma. In this retrospective study, we reviewed a large number of non-sentinel axillary node biopsies to delineate the spectrum of pathologic entities in enlarged axillary lymph nodes.

**Design:** The pathology reports of non-sentinel axillary lymph node biopsies from 1165 patients (2003-2018) were reviewed. The diagnoses were separated into 5 categories including metastatic breast carcinoma, melanoma, lymphoma, other carcinoma, sarcoma, and benign changes. The diagnostic categories were also stratified based on the gender, age, and clinical history.

**Results:** Among the 1165 patients, there were 322 men and 843 women, with a mean age of 53.7 years (median 55, range 0-97). The spectrum of diagnoses included: breast carcinoma (27.2%), lymphoma (29.5%), melanoma (3.5%), other carcinoma (3.2%), sarcoma (0.6%), and benign changes (35.3%). The most common diagnosis in men was lymphoma (62.4%), followed by benign changes (23.0%); the most common diagnosis in women was benign change (41.0%), followed by breast carcinoma (37.1%) and lymphoma (17.0%). When excluding those patients with a history of breast carcinoma, men were more likely diagnosed with lymphoma and melanoma, while women were more likely diagnosed with benign change and other carcinoma (Chi-squared, $p<0.0001$). The most common lymphoma diagnosis was diffuse large B cell lymphoma (20.1%), followed by classic Hodgkin lymphoma (14.2%), follicular lymphoma (13.4%), and small lymphocytic lymphoma (12.7%). Anaplastic large cell lymphoma accounted for only 2.6% of the lymphoma diagnoses; however, those cases were often inconspicuous and could be confused with breast carcinoma, thus requiring a high index of suspicion. Other carcinomas

were uncommon, mostly squamous cell carcinoma and poorly differentiated carcinoma. The distribution of diagnoses based on the age range and clinical history was shown in the figures.

Figure 1 – 168

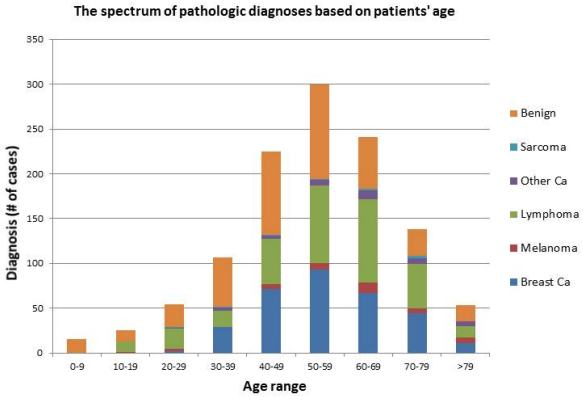
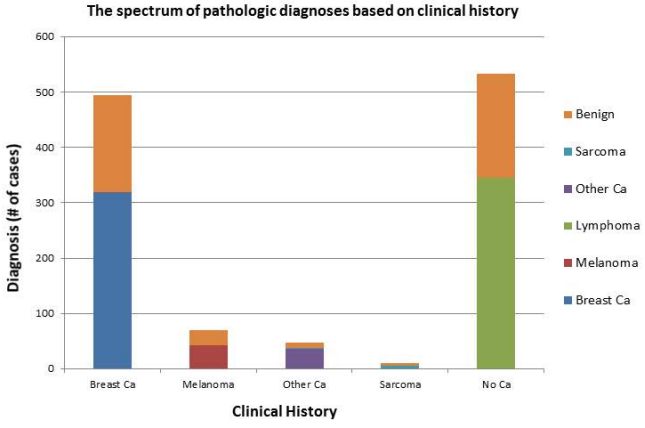



Figure 2 – 168




**Conclusions:** Through a retrospective review of a large cohort of non-sentinel axillary lymph node biopsy, we identified the spectrum of pathologic entities based on the gender, age, and clinical history, which could provide useful information for future evaluation and workup of axillary node biopsy.

## 169 Clinical Significance of Lymph Node Micrometastasis in Breast Cancer According to Tumor Biology

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**Disclosures:** J. Bryan Iorgulescu: None; Jane Brock: None

**Background:** The clinical significance of micrometastatic pN1mi (≥0.02 cm and ≤0.2 cm) disease for deciding treatment – and as a risk factor for overall survival (OS) – remains controversial in breast cancer. Registry data pre-2010 suggest a survival benefit for treating patients with chemotherapy in the presence of micrometastatic disease, but identification of occult micrometastatic disease in the NSABP-B32 cohort of node-negative patients did not correlate with clinically significant worse outcomes (*i.e.* survival differences >1-2%) that might alter treatment decisions. Herein we investigate the impact of pN1mi disease and tumor biology on survival outcomes.

**Design:** Invasive breast carcinoma patients with pathologic assessment of lymph nodes as pN0, pN1mi, or pN1a from 2004-2015 were identified from the National Cancer Database, comprising >70% of all cancers newly-diagnosed in the U.S. Exclusion criteria included prior

cancer diagnosis, neoadjuvant chemotherapy, pTis/pTX disease, and distant metastasis at presentation. Patients were stratified by pT category, biologic subtype (available as of 2010), and chemotherapy treatment, with the associated OS assessed by Kaplan-Meier and logrank techniques.

**Results:** 684,536 invasive breast carcinoma patients met inclusion and exclusion criteria, including 79.0% (n=540,514) with pN0, 4.3% (n=29,278) with pN1mi, and 16.7% (n=114,744) with pN1a disease. pN1mi 5yr-OS compared with pN0 was significantly worse across all subtypes and all T1-T3 cases in the absence of chemotherapy (Table 1). In chemotherapy-treated patients across all subtypes, there were minimal 5yr-OS absolute differences (<0.5%) between pN0 and pN1mi disease; and ≤1.0% 5yr-OS absolute differences between pN1mi and pN1a disease. pT3-4 N1mi disease behaved similarly to pT3-4 N0. Like pT1, pT2 N1mi OS differs from N0 and N1a disease.

| | | Overall, by Biologic type | | | | No Chemo | | Yes Chemo | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | 5yr-OS | p-val | 5yr-OS | p-val | 5yr-OS | p-val | |
| **ER+ HER2-** | | 304,307 | 79.6 | | | | | | | |
| | pN0 | 228,653 | 75.1 | 93.1 % | 0.08 | *92.7 %* | ***<0.001*** | 95.3 % | 0.61 | |
| | pN1mi | 18,945 | 6.2 | *92.7 %* | *ref* | *90.9 %* | *ref* | *94.9 %* | *ref* | |
| | pN1a | 56,709 | 18.6 | *90.2 %* | ***<0.001*** | *82.7 %* | ***<0.001*** | *93.9 %* | ***0.001*** | |
| **Triple Pos** | | 25,666 | 6.7 | | | | | | | |
| | pN0 | 18,303 | 71.3 | 93.3 % | 0.95 | *88.2 %* | ***0.01*** | 96.1 % | 0.83 | |
| | pN1mi | 1,586 | 6.2 | *93.7 %* | *ref* | *83.5 %* | *ref* | 95.7 % | ref | |
| | pN1a | 5,777 | 22.5 | *91.2 %* | ***0.03*** | *68.0 %* | ***0.002*** | 94.3 % | 0.12 | |
| **Triple Neg** | | 38,517 | 10.1 | | | | | | | |
| | pN0 | 30,077 | 78.1 | *86.7 %* | ***<0.001*** | *78.0 %* | ***<0.001*** | *95.3 %* | ***<0.001*** | |
| | pN1mi | 1,576 | 4.1 | *79.4 %* | *ref* | *52.3 %* | *ref* | *94.9 %* | *ref* | |
| | pN1a | 6,864 | 17.8 | *74.8 %* | ***0.001*** | *41.8 %* | ***0.003*** | *93.9 %* | ***0.02*** | |
| **ER- HER2+** | | 13,717 | 3.6 | | | | | | | |
| | pN0 | 10,114 | 73.7 | 91.0 % | 0.15 | *84.4 %* | ***<0.001*** | 93.8 % | 0.18 | |
| | pN1mi | 643 | 4.7 | *88.6 %* | *ref* | *60.2 %* | *ref* | 91.7 % | ref | |
| | pN1a | 2,960 | 21.6 | *84.9 %* | ***0.04*** | 44.5 % | 0.14 | 89.0 % | 0.09 | |
| | | **Overall, by pT** | | | | **No Chemo** | | **Yes Chemo** | | |
| | | n | % | 5yr-OS | p-val | 5yr-OS | p-val | 5yr-OS | p-val | |
| **pT0** | | 2,655 | 0.4 | | | | | | | |
| | pN0 | 2,180 | 82.1 | 89.8 % | 0.77 | 88.3 % | 0.76 | 90.3 % | 0.43 | |
| | pN1mi | 106 | 4.0 | 88.6 % | ref | 92.1 % | ref | 87.4 % | ref | |
| | pN1a | 369 | 13.9 | 92.5 % | 0.74 | 89.9 % | 0.47 | 93.5 % | 0.33 | |
| **pT1** | | 490,859 | 71.7 | | | | | | | |
| | pN0 | 418,882 | 85.3 | *91.9 %* | ***<0.001*** | *90.9 %* | ***<0.001*** | *94.5 %* | ***<0.001*** | |
| | pN1mi | 18,170 | 3.7 | *91.2 %* | *ref* | *89.0 %* | *ref* | *93.5 %* | *ref* | |
| | pN1a | 53,807 | 11.0 | *90.5 %* | ***0.02*** | *83.3 %* | ***<0.001*** | *93.2 %* | ***0.04*** | |
| **pT2** | | 171,979 | 25.1 | | | | | | | |
| | pN0 | 110,313 | 64.1 | *84.5 %* | ***<0.001*** | *78.3 %* | ***<0.001*** | *89.7 %* | ***<0.001*** | |
| | pN1mi | 9,860 | 5.7 | *83.3 %* | *ref* | *76.4 %* | *ref* | *87.4 %* | *ref* | |
| | pN1a | 51,806 | 30.1 | 82.5 % | 0.24 | *67.8 %* | ***<0.001*** | 87.3 % | 0.08 | |
| **pT3** | | 15,567 | 2.3 | | | | | | | |
| | pN0 | 7,401 | 47.5 | *77.4 %* | ***0.03*** | 65.8 % | 0.23 | 85.6 % | 0.90 | |
| | pN1mi | 1,010 | 6.5 | *82.2 %* | *ref* | *69.6 %* | *ref* | 87.6 % | ref | |
| | pN1a | 7,156 | 46.0 | *77.2 %* | ***0.01*** | *55.7 %* | ***<0.001*** | 83.6 % | 0.18 | |
| **pT4** | | 3,476 | 0.5 | | | | | | | |
| | pN0 | 1,738 | 50.0 | *57.9 %* | ***0.04*** | 45.3 % | 0.99 | 73.9 % | 0.06 | |
| | pN1mi | 132 | 3.8 | *68.3 %* | *ref* | 47.9 % | ref | *80.6 %* | *ref* | |
| | pN1a | 1,606 | 46.2 | *53.9 %* | ***0.01*** | 34.2 % | 0.36 | *72.7 %* | ***0.04*** | |
| | ***Bold*** denotes statistically significant as compared to pN1mi disease. | | | | | | | | | |

**Conclusions:** In all biological subtypes of breast cancer without adjuvant chemotherapy, pN1mi disease demonstrates significantly worse 5yr-OS than pN0. These differences are clinically substantial in the ER- and HER2+ subtypes. In patients treated with adjuvant chemotherapy, across all subtypes, the 5yr-OS differences between pN1mi and pN0 disease were negligible (<0.5%); and ≤1.0% between pN1mi and pN1a disease. In breast cancer patients of all biological subtypes that do not undergo chemotherapy, pN1mi is a clinically important prognostic factor, as compared to pN0 and pN1a disease. However, in chemotherapy-treated patients, pN1mi provides limited prognostic value.

## 170 Programmed Cell Death 1 Ligand 2 (PD-L2) protein expression is associated with better prognosis in Triple Negative Breast Cancer

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**Disclosures:** Jabed Iqbal: None; Clara Ong: None; Joe Yeong: None; Huihua Li: None; Jeffrey Chun Tatt Lim: None; Aye Aye Thike: None; Puay Hoon Tan: None

**Background:** Recent advances in immunotherapy have shown promising results in triple negative breast cancer (TNBC), defined as negative expression of oestrogen and progesterone receptors and lack of human epidermal growth factor receptor 2 (HER2) amplification. Studies regarding programmed death ligand 1 (PD-L1) and programmed cell death protein 1 (PD-1) co-inhibitory pathway observed that PD-L1 expressing TNBC had better prognosis and increased response to PD-1 checkpoint blockade. However, there are few studies on programmed cell death 1 ligand 2 (PD-L2) and its relationship to PD-L1/PD-1 co-inhibitory pathway. Hence, we aim to investigate the role of PD-L2 in the tumour microenvironment and its effect on the co-inhibitory pathway.

**Design:** Two hundred and ninety six (296) TNBC cases diagnosed between 2003 and 2013 in Singapore General Hospital were used in this study. Tissue microarray blocks (TMAs) was stained with anti-PD-L2 antibody (D7UHC) Immunostaining was scored based on expression in tumour infiltrating lymphocytes (TILS) and positivity was defined as TBX21 expression in ?1 TILs. The same cohort was

subjected to quantitative, digital gene expression NanoString assay to measure expression of a panel of 499 immune-associated genes. The cohort was divided into “mRNA PD-L2-positive” and “mRNA PD-L2-negative” based on median *PDCD1LG2* mRNA expression.

**Results:** PD-L2 expression in TILs was seen in 25.7% (72/280) cases and these patients had significantly better disease-free survival (DFS) (*p=0.0362*) and overall survival (OS) (*p=0.0379*). Multivariate analysis of PD-L2 expression in TILs, adjusted for PD-L1 protein expression, was significant in DFS (HR=0.48, *p=0.0403*) with a trend noted in OS (HR=0.44, *p*=0.058). Moreover, survival analysis of *PDCD1LG2* mRNA expression showed that mRNA PD-L2-positive patients had significantly better DFS (p=0.02) and OS (p=0.02).

**Conclusions:** Our study found that PD-L2 expression in TILs was associated with significantly better prognosis in TNBC. The data also suggests that PD-L2 could function independently of PD-L1 expression. However, further studies are required to review the prognostic signature of PD-L2 and its role in the tumour microenvironment and immune response.

## 171 T-Box transcription factors (TBX21) protein expression is associated with better prognosis in Triple Negative Breast Cancer

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**Disclosures:** Jabed Iqbal: None; Clara Ong: None; Joe Yeong: None; Huihua Li: None; Aye Aye Thike: None; Zaeen Iqbal: None; Puay Hoon Tan: None

**Background:** Triple Negative Breast Cancers (TNBC) are clinically aggressive tumours that have limited predictive biomarkers and therapeutic options. TNBCs are immunogenic tumours with a high density of tumour-infiltrating lymphocytes (TILs) that are associated with reduced distant metastasis, longer disease-free and overall survival, and are favourable prognostic markers in TNBCs. T-Box transcription factors (TBX21) is known to be a master transcription factor of Type 1 helper (Th1) T-cells. Increased TBX21 expression showed improved relapse free survival in HER2+ breast cancers, however little is known in TNBCs.

**Design:** A total of159 TNBC cases diagnosed between 2003 and 2013 in Singapore General Hospital were used in this pilot study. Tissue microarray blocks (TMAs) was stained with TBX21 antibody. Positive TBX21 expression was defined as expression of any intensity in ?20% in intratumoural tumour infiltrating lymphocytes (TILs) expression and ?38% in stromal TILs expression.

**Results:** Intratumoural TILs expression of TBX21 was 51.6% (82/159) and, TBX21 expression in stromal TILs was 50.3% (80/159). Patients with positive TBX21 expression in intratumoural TILs had significantly better disease-free survival (DFS) (*p=0.0048*) but no significant overall survival (OS) (p=0.112), while positive TBX21 in stromal TILS showed significantly better DFS and OS (*p=0.0052*, *p=0.0143*). Cox regression adjusted by age, grade, lymph node and tumour size showed significant DFS in TBX21 intratumoural TILs (HR= 0.53, 95% CI=0.28-0.98, *p=0.0421*) but found no prognostic significance of TBX21 expression in stromal TILs. In addition, TBX21 expression in intratumoural TILs reflected a significantly positive correlation with CD103, a marker for resident T cells (r=0.677, *p?0.001*), intratumoural FOXP3 expression (r=0.524, *p?0.001*), intratumoural CD38 expression (r=0.449, *p?0.001*), PD1 in TILs (r=0.447, *p?0.001*) and, PD-L1 in TILs (r=0.405, *p?0.001*). Similar correlations was observed with TBX21 stromal TILs expression.

**Conclusions:** Our study found that TBX21 expression in intratumoural TILs was associated with significantly better prognosis in TNBC and suggests that TBX21 could initiate activation of the innate immune system and hence elicit a better immune response. However, further studies are required to review its role in the tumour microenvironment and signalling pathways within TILs.

## 172 Encapsulated Papillary Carcinoma: Comparison of Clinicopathological Characteristics Associated with Frank Invasive Carcinoma

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**Disclosures:** Christopher Jackson: None; Cameron Felty: None; Kristen Muller: None

**Background:** Encapsulated papillary carcinomas (EPC) are rare breast tumors that lack myoepithelium, generally have indolent clinical behavior, and are staged as in-situ disease. A subset can be associated with frank invasion, however, this may only be discovered upon surgical excision. The clinical and pathological features of EPC associated with, and potentially predictive of, finding an adjacent conventional invasive carcinoma are largely unknown.

**Design:** A retrospective pathology database search for cases containing “papillary carcinoma” was performed from 1/2000 - 8/2018. H&E and myoepithelial immunohistochemical slides from lumpectomy and mastectomy specimens were reviewed blindly and independently by two breast pathologists. The clinicopathological features of EPC with and without an associated frank invasive component were compared.

**Results:** Twenty-two cases of EPC were identified (age 73 ± 10 years). Radiologic findings included a solid mass in all cases, 68% of which contained internal vascularity, 46% had smooth or lobulated margins, 54% showed border irregularities, and 18% had a cystic component. Half of the patients presented with a self-palpated mass, and half during screening mammography. Twelve cases contained an invasive component in the surgical excision (54.5%), which were all pT1 (0.7 ± 0.6 cm), predominantly invasive ductal or mucinous carcinoma, and low (50%) or intermediate (int) grade (50%). The invasive carcinoma was present in only 3/10 patients that underwent pre-operative diagnostic biopsy. Table 1 shows a comparison of clinicopathological features identified in EPC with and without an adjacent invasive component. Lymphovascular invasion (LVI) was found in one patient; however, no lymph node metastases were identified in 14 patients that underwent axillary staging. Adjuvant management included radiation and anti-hormonal therapy in 42% and 65%, respectively. No patients received chemotherapy. Univariate analysis found that among the factors including age, presentation, radiologic findings, EPC size, EPC grade, and presence of DCIS, only patient presentation was associated with the presence of a frank invasive component (p = 0.008).

| | EPC with invasion (n=12) | EPC without invasion (n=10) | p value |
|---|---|---|---|
| Age (years) | 71.8 ± 12.5 | 74.4 ± 8.8 | 0.6 |
| Self-palpated mass | 10/12 (83%) | 2/8 (25%) | 0.008 |
| Irregular borders | 7/12 (58%) | 5/10 (50%) | 1.0 |
| EPC size (cm) | 1.8 ± 1.1 | 1.5 ± 0.9 | 0.5 |
| EPC grade int/high | 6/12 (50%) | 6/10 (60%) | 1.0 |
| DCIS present | 9/12 (75%) | 7/10 (70%) | 1.0 |

**Conclusions:** In our cohort, patients with EPC who presented with a self-palpated mass were more likely to have frank invasion, and the majority of invasive carcinomas were found only in the surgical excisions. EPC nuclear grade and tumor size were not associated with invasion.

## 173 Fibroepithelial Neoplasms in the Pediatric and Adolescent Population: A Clinicopathological Study of 58 Cases

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**Disclosures:** Elizabeth Jacobi: None; Ninad Patil: None; Ekene Okoye: None; Nour Sneige: None

**Background:** Breast neoplasms in the pediatric and adolescent population are rare and the vast majority are benign. Fibroepithelial neoplasms (FEN) are the most prevalent, which include fibroadenoma (FA), juvenile fibroadenoma (JF), cellular fibroadenoma, tubular adenoma, and phyllodes tumor (benign, borderline, and malignant). Although the histologic features of each are defined, there exist many lesions with overlapping features making exact categorization challenging. We undertook this study of 58 cases of pediatric and adolescent FENs to evaluate various histopathologic features that may help characterize these neoplasms and predict clinical outcome.

**Design:** The institutional database was searched from 2006-2017 for excision specimens of FENs in females less than or equal to 18 years of age using key words “phyllodes”, “JF”, “FA”, and “fibroepithelial”. Clinical information was obtained from the patients’ charts and pathology reports were reviewed to document macroscopic features. H&E slides were reviewed by 4 pathologists and the following parameters were recorded: margin status, borders, mitoses per 10 hpf, necrosis, cellular pleomorphism, stromal cellularity, and stromal pattern (Table 1).

**Results:** We identified 58 excision specimens of FENs, including seven cases from 1988-2004, in 50 female patients ranging from 10 to 18 years of age (mean=15 years). FENs ranged in size from 0.9 to 22 cm (mean=5.9 cm). On review, FENs were categorized as pure FA (24%, 14/58), JF (47%, 27/58), JF with less than 50% phyllodes tumor (12%, 7/58), benign phyllodes (12%, 7/58), borderline phyllodes (5%, 3/58), and malignant phyllodes (0%, 0/58). Positive resection margins were seen in 59% (34/58) of cases. Thirty patients had no documented clinical follow up. Duration of follow up for the remaining 32 cases ranged from less than 1 month to 120 months (mean=13.6 months) with no documented recurrences. See Table 1.

Table 1. Histologic parameters of FEN

| Parameter Evaluated | FA<br>n (%) | JF<br>n (%) | JF with <50% Phyllodes<br>n (%) | Benign Phyllodes<br>n (%) | Borderline Phyllodes<br>n (%) |
|---|---|---|---|---|---|
| Total<br>*58 cases* | 14 (24) | 27 (47) | 7 (12) | 7 (12) | 3 (5) |
| Margin Status | 12 (86) | 11 (41) | 5 (71) | 4 (57) | 2 (67) |
| *Positive* | 2 (14) | 15 (56) | 2 (29) | 3 (43) | 1 (33) |
| *Negative* | - | 1 (3)* | - | - | - |
| *Cannot determine* | | | | | |
| Border | 14 (100) | 27 (100) | 7 (100) | 5 (71) | 1 (33) |
| *Well Circumscribed* | - | - | - | 2 (29) | 2 (67) |
| *Infiltrative* | | | | | |
| Mitoses per 10 hpf | 14 (100) | 26 (96) | 7 (100) | 3 (43) | 1 (33) |
| *< 4* | - | 1 (4) | - | 3 (43) | 2 (67) |
| *4 – 10* | - | - | - | - | - |
| *> 10* | - | - | - | 1 (14)** | - |
| *Cannot determine* | | | | | |
| Necrosis | - | - | - | 1 (14) | - |
| *Present* | 14 (100) | 27 (100) | 7 (100) | 6 (86) | 3 (100) |
| *Absent* | | | | | |
| Cellular Pleomorphism | 13 (100) | 27 (100) | 7 (100) | 7 (100) | - |
| *Minimal* | - | - | - | - | 3 (100) |
| *Moderate* | - | - | - | - | - |
| *Marked* | | | | | |
| Stromal Pattern | 13 (100) | 27 (100) | 4 (57) | 4 (57) | - |
| *Homogenous* | - | - | 3 (43) | 3 (43) | 3 (100) |
| *Heterogenous* | - | - | - | - | - |
| *Overgrowth* | | | | | |
| Follow Up (months) | 4 (31) | 6 (23) | - | 3 (43) | 1 (33) |
| *< 12* | 1 (8) | 3 (11) | 2 (29) | 2 (29) | 2 (67) |
| *12 – 24* | 2 (15) | 9 (33) | 1 (14) | 1 (14) | - |
| *> 24* | 6 (46) | 9 (33) | 4 (57) | 1 (14) | - |
| *None* | | | | | |

*Specimen fragmented

**Extensive coagulative necrosis present

**Conclusions:** The vast majority of FENs in the pediatric and adolescent population are benign. In our review the most common FEN was JF. Various histologic patterns were observed with overlapping features. However, recurrence rate after resection, including resections with

positive margins, is extremely low. Therefore, despite seemingly worrisome macroscopic and microscopic features, a conservative management approach is warranted.

## 174 Association between Histologic Features of Atypical Ductal Hyperplasia and Risk of Breast Cancer

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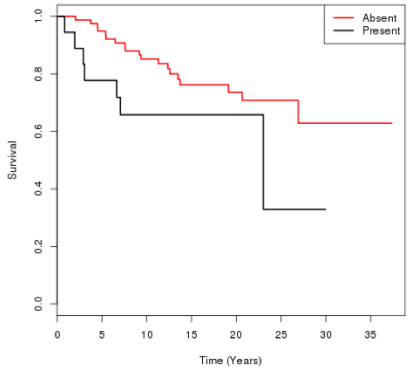
**Disclosures:** Khadijeh Jahanseir: None; Amy Degnim: None; Daniel Visscher: None; Robert Vierkant: None; Derek Radisky: None; Jodi Carter: None; Teresa Allers: None; Marlene Frost: None; Mark Sherman: None; Stacey Winham: None

**Background:** Atypical ductal hyperplasia (ADH) is characterized by a morphologically heterogeneous intraductal proliferation of mammary duct epithelial cells. The significance of the different histologic features of ADH in the long term risk of breast cancer (BC) is undefined. We investigated the association between different histological features of ADH with the risk of subsequent BC.

**Design:** Excisional breast biopsies of a subset of women with ADH from our institute Benign Breast Disease Cohort (N=99) were evaluated for histologic features of ADH including archetictural patterns, stromal reaction (increased number of fibroblsts surrounding the duct), chronic inflammation, non-monomorphous nuclear cytology, mitosis, individual cell necrosis (ICN), stromal fibrosis and the background lesion. Subsequent BC events, both invasive cancer and ductal carcinoma in situ, were obtained via patients' records. Women were followed from date of ADH diagnosis to BC, death or last follow-up. Associations of histologic features with BC risk were assessed using Kaplan-Meier (KM) curves and age-adjusted Cox proportional hazards regression models.

**Results:** Among the 99 women with ADH, 46 (45.5 %) had only cribriform, 17 (16.8 %) had only micropapillary and 35 (34.7 %) had both micropapillary and cribriform architecture. One was considered unclassified. Stromal reaction was found in 18 (18.2%), chronic inflammation in 27 (27.3%), non-monomorphous cytology in 42 (42.4%), mitosis in 5 (5.1%), ICN in 59 (59.6%), stromal fibrosis in 24 (24.2%), background of papilloma in 21 (21.2%), background of radial scar in 13 (13.1%), background of columnar cell change in 11 (11.1%) and background of flat atypia in 8 (8.1%). The mean follow-up was 14.7 y, median was 13.5 y. A total of 26 women (26.3 %) developed BC. Presence of stromal reaction was associated with an increased risk of BC after adjustment for age (HR 2.75, 95% CI 1.13-6.71, p=0.03). We also observed an increased BC risk for women with both micropapillary and cribriform architecture (p=0.32) and for those with non-monomorphous nuclear cytology (p=0.10), but neither reached statistical significance.

Figure 1 – 174




Breast cancer-free survival by stromal reaction

**Conclusions:** We observed variable histologic features among women with ADH; stromal reaction was one factor associated with increased risk of BC. This suggest that an interaction with host environment promotes BC development in women with ADH. Further investigation is needed to understand mechanisms that might be associated with these morphologic features and risk of BC.

## 175 Histopathologic predictors of residual disease in concurrent additional margin excisions in-spite of negative primary breast resection margins

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**Disclosures:** Judith Jebastin Thangaiah: None; Nilesh Gupta: None; Absia Jabbar: None; Daniel Schultz: None; Dhananjay Chitale: None

**Background:** Negative pathological margin status is an important component of optimal management in breast cancer to minimize local recurrence, but there is lack of consensus regarding optimal negative margin width. In our institution, concurrent additional margins are frequently submitted for final pathologic evaluation along with breast resection specimens. Our aim of this study was to study frequency of residual disease and association of clinicopathologic parameters that may help predict presence of residual disease in the concurrent additional margins when primary resection specimen had negative margins (no ink on tumor).

**Design:** Over a 2 year period, we selected patients whose primary resection margins were negative from all consecutive cases of surgically resected breast specimens with concurrent additional surgical margins submitted for pathologic examination. We compared pathologic features of patients with ("study group") versus without ("control group") residual disease in the concurrent additional margins. Pathologic features abstracted from final diagnostic report included CAP checklist elements, histological subtypes, margin status in addition to other relevant clinicopathologic findings. Statistical analyses with $p<0.05$ two-tailed level of significance were performed using Chi square, Fisher exact, t-test or Mann-Whitney U tests as appropriate; we used a Cox proportional hazards model for multivariate analysis.

**Results:** Out of 519 cases that satisfied above criteria, 8.5% (44/519) cases had residual disease in concurrent additional margin excisions. 95 women contributed to this case control study, 51 control group, 44 study group. Primary diagnoses and other data for all cases are listed in Table 1. By univariate analysis, invasive lobular carcinoma (p=0.03), extensive intraductal component (EIC) (p=0.004), LCIS (p=0.02), Her2 status (p=0.0008) were statistically significant between the two groups. Multivariate analysis showed EIC as the most statistically significant parameter.

| | |
|---|---|
| Primary Diagnosis | |
| Ductal carcinoma in-situ | 3 |
| Invasive ductal carcinoma, NOS | 70 |
| Invasive lobular carcinoma | 8 |
| Mixed ductal and lobular carcinoma | 9 |
| Mucinous carcinoma | 4 |
| Solid papillary carcinoma | 1 |
| Focality | |
| Unifocal | 63 |
| Multifocal | 29 |
| Not applicable (DCIS cases) | 3 |
| AJCC staging | |
| pStage 1 | 72 |
| pStage 2 | 18 |
| pStage 3 | 1 |
| pStage 4 | 1 |
| Tis | 3 |
| Estrogen Receptor status | |
| Negative | 13 |
| Positive | 81 |
| Not available | 1 |
| Her2 status | |
| Negative | 58 |
| Positive | 16 |
| Equivocal | 8 |
| Not available | 13 |
| LCIS | |
| Absent | 83 |
| Present | 12 |

**Conclusions:** 61% of histologically unicentric tumors with negative primary resection margin, showed residual breast cancer in concurrent additional margins. Invasive lobular carcinomas, EIC, Her2 status, LCIS were the most significant pathologic parameters that predicted residual tumor in univariate analysis. Interestingly, width of negative margin and tumor size did not correlate with residual disease.

## 176 A Comparison of GATA3, HMWK, AE1/AE3, CAM 5.2, CD34, p63 as markers for breast spindle metaplastic cell carcinoma and its mimics

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**Background:** Spindle cell lesions of the breast represent rare entities; However, accurate diagnosis and classification of breast spindle cell lesions remain challenging especially in core biopsies. The difficulties lies in the overlap of clinical, radiological, morphologies and immunohistochemical. Previously, several reports have investigated the value of immuno panel in aiding the diagnosis. The role of GATA 3 as a highly specific marker in breast carcinoma has been well established. However, very few studies have addressed the value of GATA3 in metaplastic breast carcinoma with spindle differenation. In the present study, we evaluated cases of spindle cell lesions of the breast with different diagnoses (reactive, benign, and malignant). We discussed the immunopanel especially GATA3 in the correction identification of metaplastic carcinomas with spindle cell differentiation.

**Design:** A total of 78 patients who underwent breast core biopsies or breast resections at Northwell Health between 2010 and 2018, and who were diagnosed with benign and malignant tumor/tumor-like lesions that had spindle cell components following the histopathological examination were included in the study. The differential diagnosis include angiosarcoma, nodular fasciitis, fibromatosis, myofibroblastoma, phyllodes tumors (benign, boardline and malignant) and metaplastic carcinoma with spindle cell differentiation. The immunohisotchemisry was also performed including HMW, CAM5.2, AE1/AE3, P63, CD34 and GATA 3.

**Results:** The results was summarized in Table 1. Compared to the mimics, GATA 3 expression was significantly higher in metaplastic carcinomas (88.2% vs 1.6% *p<0.001*). In contrast, GATA 3 was only found in one angiosarcoma case. The sensitivity and specificity for detecting metaplastic carcinomas reached (88.2% and 98.4%). The staining patterns varied from weak patch to strong diffuse. Regarding cytokeratin panels, none of the three individual cytokeratin is as sensitive or specific as GATA 3 for metaplastic carcinomas.

| Immunos | AS (n=8) | FM (n=8) | MF (n=12) | NF (n=6) | Benign PT (n=13) | Borderline PT (n=9) | Malignant PT (n=5) | MT (n=17) |
|---|---|---|---|---|---|---|---|---|
| **GATA 3** | 1/8 | 0/8 | 0/12 | 0/6 | 0/13 | 0/9 | 0/5 | 15/17 |
| **HMWCK** | 0/8 | 0/8 | 0/12 | 0/6 | 0/13 | 0/9 | 0/5 | 9/17 |
| **AE1/AE3** | 0/8 | 0/8 | 0/12 | 0/6 | 0/13 | 0/9 | 0/5 | 8/17 |
| **CAM5.2** | 0/8 | 0/8 | 0/12 | 0/6 | 0/13 | 0/9 | 0/5 | 10/17 |
| **P63** | 0/8 | 0/8 | 0/12 | 0/6 | 0/13 | 0/9 | 0/5 | 7/17 |
| **CD34** | 8/8 | 0/8 | 12/12 | 0/6 | 12/13 | 7/9 | 3/5 | 0/17 |

AS= Angiosarcoma; FM= Fibromatosis; MF= Myofibroblastoma; NF= Nodular fasciitis; PT= Phyllodes tumor; MT= Metaplastic carcinoma

**Conclusions:** GATA 3 is a specific and sensitive marker for identifying metaplastic carcinoma with spindle cell differentiation.

## 177 Outcomes of Benign Intraductal Papillomas Diagnosed on Core Needle Biopsy: Immediate Surgical Excision Versus Clinical Follow-up

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**Disclosures:** Zhongbo Jin: None; Jaya Asirvatham: None; Nada Al Qaysi: None

**Background:** Atypical papillomas diagnosed on core needle biopsy (CB) are excised, as the risk of associated malignancy is high. The management of benign intraductal papilloma (BIP) is controversial due to varying rates of upgrade reported on excision average (7%). This study was designed to compare the outcomes between BIPs that were immediately excised (within 6 months) versus those that were followed up for at least 24 months at our institution.

**Design:** An electronic data base search (keyword intraductal papilloma) identified 192 BIP diagnosed by CB between 2010 to 2016, of which 127 were excised and 65 were followed. Clinical practice during this time frame was stable, with one surgeon prefering to excise all BIP and the other prefering to clinically follow up all radiologically concordant BIP. Cases with concurrent atypia/malignancy in the same

quadrant were excluded. Incidental papillomas were included. Preliminary data after chart and slide review of all CB meeting inclusion criteria and subsequent excisions of BIP from 2014 &2015 are summarized below.

**Results:** 48 BIP from 48 female patients were analyzed. All were radiologically concordant. 17 BIP were clinically followed (mean age: 54.3; mean size on imaging: 8.7 mm), with an average of 2.8 visits (mean: 40 months; range 32- 55). 1 patient upgraded to invasive ductal carcinoma in the same quadrant after 49 months. 31 underwent excision (mean age: 53.7 years; mean size on imaging: 8.8 mm). Residual papilloma was present in 68% (21/31). ADH was present on excision in 3 cases (2 within papilloma, 1 in adjacent breast tissue). Of these, 2 patients remained stable on follow up imaging. The other developed a new atypical papilloma and invasive carcinoma 25 and 30 months after the initial diagnosis of BIP respectively, in the same quadrant. There were no statistically significant differences between the two groups. Both patients who had developed invasive ductal carcinoma had a strong family history of breast cancer (multiple family members).

**Conclusions:** The majority of radiologically concordant BIP were not upgraded to malignancy on excision or clinical follow-up. Clinical follow up may be a reasonable alternative for radiologically concordant BIP, in patients without a personal history of carcinoma. Surgical management or close clinical follow up may be considered for patients with a strong family history of breast cancer.

## 178 Incidental Atypias and Cancers in Breast Reductions: What Happens Next?

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**Disclosures:** Amandeep Kaur: None; Megan Sullivan: None

**Background:** Finding a previously undetected breast cancer (BC) in a reduction specimen is an uncommon event, with a reported incidence of <1-5%. When discovered, these cancers can be clinically difficult to manage as margin status cannot be determined. Besides BC, other non-obligate precursor lesions such as atypical ductal hyperplasia (ADH) and lobular neoplasia (LN) are diagnosed in these specimens. While pathologists make these diagnoses, what happens next for the patient is highly variable. In this study, we looked at patients with incidentally discovered atypias and/or cancers in reductions with a focus on the subsequent follow-up.

**Design:** After IRB approval, the pathology database was searched for women who had a reduction with a diagnosis of invasive carcinoma (IC), ductal carcinoma in situ (DCIS), ADH, or LN between January 1, 2000 and July 1, 2018. Patients with concurrent ipsilateral BC were excluded. Clinical information was collected from the EMR including: age, personal history of BC, subsequent surgical and/or medical interventions and breast imaging.

**Results:** There were 5208 total reductions; 73 (1.4%) had an incidental finding. 37 (51%) had LN as the most significant finding, 10 (14%) ADH, 16 (22%) DCIS and 10 (14%) IC. The average age was not different between the groups, and ranged from 55.4 for ADH to 57.1 for LN. 20 patients (27%) underwent mastectomy, 7 (35%) of whom had a history of BC (Table 1). Residual cancer was present in 4 mastectomies (4/13; 31%).

20 patients who did not undergo surgery had a history of BC (38%). Of those with no history of BC, 8 got hormonal therapy (7 LN, 1 ADH). Subsequent imaging was available for 28 patients (average 87 months; range 5-180 months). 3 patients developed a subsequent BC in the same breast: one patient with ADH developed DCIS after 2 years, one patient with DCIS developed IC after 6 months and one patient with LCIS developed DCIS after 7 years.

Table 1: Patients who underwent additional surgery

| Reduction diagnosis | Total patients | Personal history of BC | Size of lesion | Type of surgery | Upgrade |
|---|---|---|---|---|---|
| LN | 2/37 (5.4%) | 1 | N/A | 2 Mastectomy (one bilateral) | None |
| ADH | 3/10 (30%) | 3 | N/A | All Mastectomy | 1 (DCIS) |
| DCIS | 6/16 (37%) | 2 | 0.4-2.2 cm | 6 Mastectomy (5 bilateral) | None |
| IC | 7/10 (70%) | 1 | <0.5-5.5 cm | 6 Mastectomy (5 bilateral, one with SLN)<br>1 Lymph node dissection alone | 1 (+SLN) |

**Conclusions:** In 17 years, incidental findings were present in 1.4% of reductions; 36% were DCIS or IC. 27% of patients had a mastectomy, including 4 who only had LN or ADH in their reduction. Importantly, the 2 patients whose reductions were diagnosed as “ADH bordering on DCIS” opted for mastectomy, demonstrating the impact of diagnostic wording on decisions. 11% were upgraded at surgery. Of those with BC in their reduction, 31% had additional tumor in the mastectomy. 11% of patients with only clinical follow-up were diagnosed with an ipsilateral BC, 6 months to 7 years after their reduction.

## 179 Atypical Ductal Hyperplasia and Those Boarding on Ductal Carcinoma in Situ Should be Included in the Active Surveillance Clinical Trials

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**Disclosures:** Thaer Khoury: None; Nashwan Jabbour: None; Xuan Peng: None; Li Yan: None; Marie Quinn: None

**Background:** Low grade mammary ductal lesions are subjectively divided into atypical ductal hyperplasia (ADH), ADH bordering on ductal carcinoma in situ (DCIS) and DCIS. Patients with either ADH or ADH bordering on DCIS, unlike DCIS, are paradoxically denied the eligibility for active surveillance (AS) clinical trial. We hypothesize that patients with ADH or ADH bordering on DCIS have lower risk of upgrade rate and should be included in the AS clinical trials.

**Design:** We applied the clinical inclusion criteria of the COMET clinical trial on our patients who were mammographically screened between 2006 and 2017. We identified a total of 228 candidate cases with reported diagnosis of ADH (n=153), ADH bordering on DCIS (n=20), or low grade DCIS (n=55). These cases were re-classified dichotomously to ADH (n=174) vs. DCIS (n=54) using Page's criteria. The following histologic findings on the core needle biopsy (CNB) were recorded, lesion size, number of involved cores, percentage of necrosis occupying the duct (should be ≤30%), and whether the ducts were completely or partially involved. We used 30% cutoff to define comedo vs. focal necrosis. We included these covariates along with patients' age to calculate the risk of residual high risk (HR) disease in the excisional biopsy (EB). The EB slides were reviewed. Upgrade to high HR residual disease was identified when any of these lesions were found in the EB: invasive carcinoma (IC), high grade DCIS, or DCIS with comedo necrosis.

**Results:** There were 20 (8.8%) cases with upgrade to HR disease, 1 (5%) high-grade DCIS, 11 (55%) IC, and 8 (40%) DCIS with comedo-necrosis. There were 4 (2.6%) reported ADH cases and 9 (45%) reported ADH bordering on DCIS cases which were reclassified to DCIS using Page's criteria. There were 11 (55%) reported ADH bordering on DCIS cases and 14 (25.5%) reported DCIS cases which were reclassified to ADH using Page's criteria. The upgrade rate to HR disease was 9 (5.2%) of reclassified ADH cases vs. 9 (16.7%) of reclassified DCIS ($p=0.017$); and 4 (2.6%) of reported ADH vs. 3 (15%) reported ADH bordering on DCIS vs. 11 (20%) reported DCIS ($p<0.001$). None of the histologic variables predicted upgrade to HR disease in the reclassified DCIS cases. However, when all cases (n=228) were included in the analysis, complete involvement of the ductal space predicted upgrade to HR disease (Odds ratio 12.4, $p=0.018$).

**Conclusions:** ADH and ADH bordering on DCIS have lower upgrade rate than DCIS. We recommend opening an AS clinical trial for these patients.

## 180 Low TOP3B level associated with CDKN1B overexpression is linked to tamoxifen resistance in breast cancer: gene set enrichment analysis

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**Disclosures:** Dong-Hoon Kim: None; Kyueng-Whan Min: None; Seoung Wan Chae: None; Sung-Im Do: None; Young-Ha Oh: None; Byoung Kwan Son: None

**Background:** Estrogen receptor (ER)-positive breast cancers are generally treated with tamoxifen. As a safety device for cell division, CDKN1B has been associated with a variety of drug therapy responses. It has been suggested that CDKN1B plays an important role to determine a breast cancer patient's survival, but the precise mechanism is not fully understood.

**Design:** This study included 204 cases of breast cancer from Kangbuk Samsung Medical Center (KBSMC), with 1353 cases from the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) database serving as a validation cohort. The correlations between CDKN1B expression and clinicopathological factors and patient outcomes were analyzed.

**Results:** Low CDKN1B expression was associated with worse disease-free survival (DFS) and overall survival (OS) in ER-positive breast cancer patients receiving tamoxifen in the KBSMC and METABRIC cohorts (all $p < 0.05$). Using gene set enrichment analysis, we found genes (IDH2, SNX29, CACNA1H, TAOK2, and TOP3B) that were linked to low CDKN1B expression. The identified genes had oncogenic propensity as well as drug resistances and showed a negative correlation with CDKN1B expression.

Figure 1 – 180

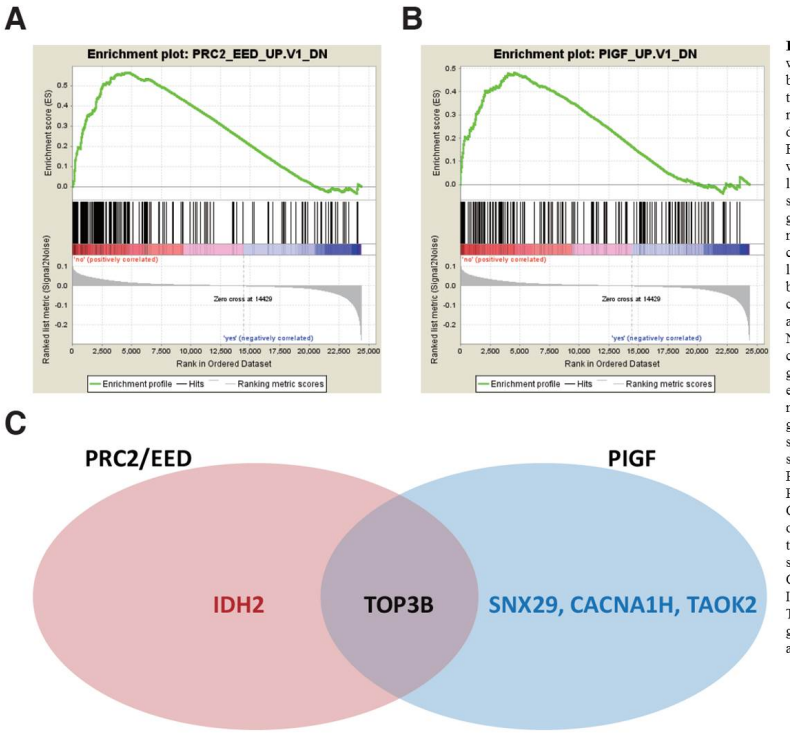


**Figure 1**. The gene expression data was analyzed using GSEA to extract biological knowledge. In every thumbnail, the green curve represents the evolution of the density of the genes identified in the RNA-seq. GSEA calculates these by walking down the ranked-ordered list of genes, increasing a running-sum statistic when a gene is in the gene set and decreasing it when it is not. The top of this list (red color) contains genes upregulated in 'no' as low CDKN1B expression. The bottom of the list (blue color) contains downregulated genes in 'yes' as high CDKN1B expression. The NES (Normalized Enrichment Score) computes the density of modified genes in the dataset with the random expectancies, normalized by the number of genes found in a given gene cluster, to take into account the size of the cluster. These figures show enrichment of the (A) PRC_EED_UP.V1_DN and (B) PIGF_UP.V1_DN in 'no' as low CDKN1B expression. Bottom, value of the ranking metric along the list of the ranked genes. Venn diagram showing the distribution of CDKN1B-related gene (TOP3B, IDH2, SNX29, and CACNA1H and TAOK2) of PRC2/EED and PIGF gene set using gene set enrichment analysis (GSEA) in METABRIC

Figure 2 - 180

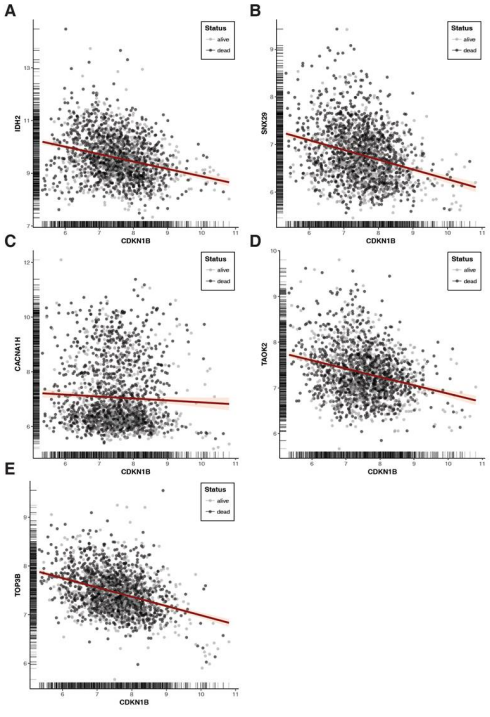


**Figure 2.** Scattered plot and regression line demonstrating relationship between CDKN1B and identified genes. CDKN1B gene expression is inversely correlated to the TOP3B, IDH2, SNX29, and CACNA1H and TAOK2 in METABRIC

**Conclusions:** Target therapy for the genes identified in this study that were related to low CDKN1B expression may improve therapeutic efficacy in ER-positive breast cancer with tamoxifen resistance.

## 181 High UTX expression is associated with tamoxifen resistance in luminal A breast cancer

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**Disclosures:** Dong-Hoon Kim: None; Kyueng-Whan Min: None; Seoung Wan Chae: None; Sung-Im Do: None;Young-Ha Oh: None; Byoung Kwan Son: None

**Background:** High Ubiquitously transcribed tetratricopeptide repeat, X chromosome (UTX) expression could associated with invasion of tumor cells in in vivo study. It has been suggested that UTX plays an important role to determine a breast cancer patient's survival, but the precise mechanism is not fully understood.

**Design:** The study enrolled 224 breast cancer patients from Kangbuk Samsung Medical Center. Nuclear UTX and cytoplasmic MMP-11 expression were evaluated using immunohistochemistry of tumor tissue microarray specimens. We analyzed the relationships between the expression of UTX and clinicopathological parameters.

**Results:** High UTX expression was significantly associated with worse clinical outcomes such as high histologic grade and lymphovascular invasion (all $p < 0.05$). Furthermore, overexpression of MMP-11, matrix metalloproteinase related to tumor invasiveness was linked to high UTX expression ($p=0.013$). High UTX expression was associated with poor overall survival and disease-free survival (DFS) in the patients with luminal A breast cancer receiving tamoxifen (all $p < 0.05$).

Figure 1 – 181

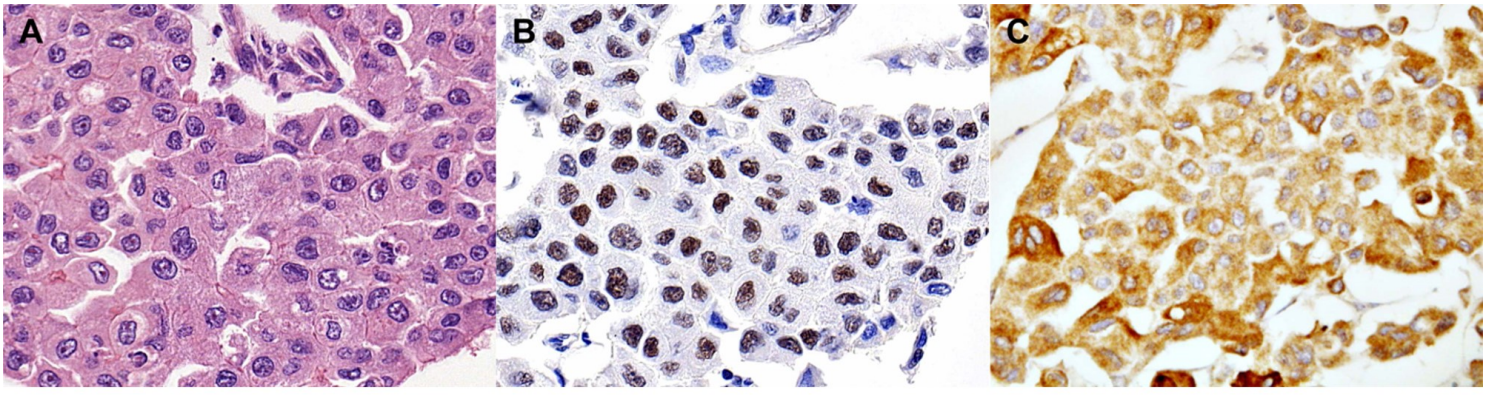


Fig1. Representative case (A) showing nuclear UTX expression (B) and cytoplasmic MMP-11 expression (C). (Original magnification, x400)

Figure 2 - 181

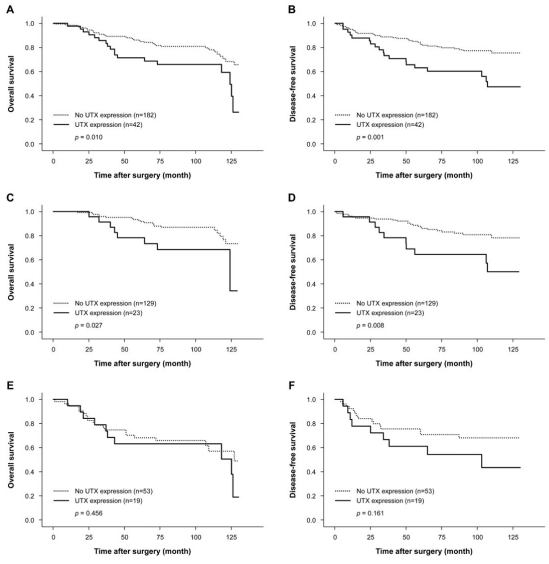


Fig 2. Kaplan–Meier plots for overall survival (A, C, E) and disease-free survival (B, D, F) in breast cancers according to UTX expression (log-rank test). A and B, Kaplan–Meier plots for UTX expression in all 224 patients; C and D, Kaplan–Meier plots for UTX expression in patients with luminal type breast cancer; E and F, Kaplan–Meier plots for UTX expression in patients with non-luminal type breast cancer.

**Conclusions:** UTX could provide an important indicator for determining therapeutic plan in luminal A breast cancer.

## 182 SOX10 as a Marker of Basal-Like Breast Cancer

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**Disclosures:** Kristina-Ana Klaric: None; Karama Asleh: None; Xiu Qing Wang: None; Tadros Atalla: None; Sarah Strickland: None; Torsten Nielsen: *Consultant*, NanoString Technologies, Inc.; *Consultant*, Bioclassifier LLC; Zuzana Kos: None

**Background:** Basal-like breast cancer is a particularly aggressive molecular subtype that is more prevalent in younger women and associated with early relapse. Most cases lack expression of ER, PR and HER2, which limits targeted therapeutic options. Beyond triple-negative phenotype, basal-like breast cancer is defined by expression of genes found in the outer/basally-located epithelial layer of mammary glands, such as cytokeratins 5, 14 and 17 and epidermal growth factor receptor. Triple-negative tumours that express basal markers are associated with worse survival than triple-negative tumours that lack basal-like markers. SOX10, a readily available immunostain currently used as a marker of melanocytic differentiation, has also been shown to be expressed in a proportion of breast cancers and has recently been reported to be preferentially expressed in triple-negative and metaplastic carcinomas. In this study, we sought to assess the association of SOX10 expression with intrinsic molecular subtype as defined by PAM50 gene expression.

**Design:** SOX10 immunostaining was performed on tissue microarrays constructed from formalin-fixed paraffin embedded blocks, enriched for ER-negative and ER-weakly positive (Allred 3-5) breast cancers from 2 previous studies (conducted 2008-2013). PAM50 intrinsic subtype was determined for each tumour using RT-qPCR. Staining in >1% of cells was considered SOX10 positive. Clinicopathologic data were extracted by chart review.

**Results:** A total of 216 cases were informative for both SOX10 IHC and PAM50 subtype, including: 106 basal-like, 68 HER2-enriched, 31 Luminal B and 11 Luminal A cancers. SOX10 was positive in 76/106 (71.7%) basal-like breast cancers but only 3/110 (2.7%) other subtypes (all HER2-enriched) ($p<0.01$); resulting in a sensitivity of 71.7% and specificity of 97.3%. No cancers classified as Luminal stained for SOX10. These results are independent of receptor status. In the selectively enriched cohort of weak ER positive tumours (n= 57), 27 (47.3%) were classified as basal-like by PAM50, of which 19 (70.4%) stained for SOX10. None of the non-basal-like tumours in this weak ER positive cohort stained for SOX10.

**Conclusions:** SOX10 IHC is a sensitive and highly specific marker for the basal-like intrinsic subtype. Unlike other IHC basal-like biomarkers commonly used in clinical practice, it is independent of receptor status. In the setting of weakly ER positive breast cancers, SOX10 can inform on tumour biology and prognosis.

## 183 A Correlation of Oncotype DX Breast DCIS Score with the Mean Number of Touching Tumor-Infiltrating Lymphocytes per Duct

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**Disclosures:** Miglena Komforti: None; Susan Fineberg: *Advisory Board Member*, Genomic health; Bryan Harmon: None

**Background:** Presence of dense tumor-infiltrating lymphocytes (TILs) in invasive breast cancer has been associated with improved survival and response to neoadjuvant chemotherapy, particulalrly in triple negative and HER2+ tumors. Recent research suggests that the number of TILs immediately surrounding a DCIS duct or away from it by one lymphocyte thickness, called touching TILs, can be reproducibly quantified and is related to DCIS recurrence risk.[1] We quantify and correlate touching TILs with Oncotype DX Breast DCIS Score (ODX).

**Design:** Forty-six ER+, <25mm DCIS cases with ODX were available for review. As per guidelines[1], 6 cases with DCIS forming a mass ≥ 5mm or only a single large DCIS duct were excluded. As per guidelines,[1] TILs touching or within one lymphocyte cell thickness from ducts' basement membrane were counted, up to 20 ducts. The mean number of touching TILs per DCIS duct was calculated as absent/very scanty (0-5), sparse (6-20), and dense (>20). ODX was reported as low (<39), intermediate (39-54), and high (≥55). Information on age, nuclear grade, necrosis, and progesterone receptor (PR) expression was obtained from pathology reports .

**Results:** Of 40 cases scored, 28 (70%) had low ODX and 12 (30%) had intermediate/high ODX; and, 30 (75%) demonstrated absent/very scanty touching TILs, while 7 (17.5%) showed sparse and 3 (7.5%) dense touching TILs (Table 1). Cases with absent/very scant touching TILs had a lower average ODX (mean 20.3) than cases with sparse or dense touching TILs (mean 43.8)(p= 0.011). There was a statisitcally signficant association between absent/very scanty touching TILS and low ODX DCIS Score (p=.0411), absent necrosis (p=.0026) and positive PR expression (p=.0121) ( Table1). There was no significant association between age or nuclear grade and density of touching TILS, however our sample only included 5 patients <50 years old and 5 cases with low nuclear grade. (Table1)

Table 1: Correlation of Touching Tumor-Infiltrating Lymphocytes in DCIS with Oncotype Score, Necrosis, PR Expression, and Nuclear Grade

| | Absent/scanty touching TILs (≤5) | Sparse or dense touching TILs (≥6) | p-value |
|---|---|---|---|
| Low ODX | 24 | 4 | 0.0411 |
| Intermediate or high ODX | 6 | 6 | |
| Necrosis | 14 | 10 | 0.0026 |
| No necrosis | 16 | 0 | |
| PR positive | 30 | 7 | 0.0121 |
| PR negative | 0 | 3 | |
| Nuclear grade 1 | 5 | 0 | 0.3059 |
| Nuclear grade 2 or 3 | 25 | 10 | |

Legend: DCIS-ductal carcinoma in situ; ODX-Oncotype DxDCIS Score; TILs-tumor-infiltrating lymphocytes; PR-progesterone receptor

*Note: All DCIS cases are <25mm in size and express estrogen receptor (ER+)

**Conclusions:** We demonstrate a significant association between Oncotype DX DCIS Score and density of touching TILs in DCIS. We also observed significant associations of density of touching TILs with PR status and presence of necrosis. These results support the findings of Toss and colleagues who reported association between touching TILS in DCIS and recurrence risk .[1] Quantification of touching TILs may provide prognostic information in DCIS.

**Reference:** 1.Toss MS, et al, Prognostic significance of tumor-infiltrating lymphocytes in ductal carcinoma in situ of the breast. Modern Pathol 2018;31:1226-36

## 184 The Role of FGFR4 with Trastuzumab Resistance in HER2/neu-Amplified Breast Cancer

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**Disclosures:** Miglena Komforti: None; Joseph Albanese: None; Yungtai Lo: None

**Background:** Despite the clinical efficacy of HER2-targeted therapy, many patients exhibit innate resistance or acquire resistance after therapy. Herein, we evaluate the relationship between FGFR4 and HER2/neu amplification in invasive mammary carcinoma and breast

cancer cell lines, respectively. In the same cohort, we also evaluate the association between FGFR4 amplification and resistance to trastuzumab therapy.

**Design:** A pilot study identified 28 patients with HER2-positive (HER2+) invasive breast cancer, who were also on trastuzumab therapy. Immunohistochemical (IHC) analysis for FGFR4 in all tumors has been performed. FGFR4 positivity (FGFR4+) was defined as the presence of at least moderate cytoplasmic and/or membranous expression in ≥10% of invasive carcinoma cells. Furthermore, 5 breast carcinoma cell lines were analyzed for simultaneous HER2/neu and FGFR4 amplification on Western blot. Resistance to trastuzumab was defined as recurrence after adjuvant therapy, failure to achieve pathological complete response after neoadjuvant therapy, or progression within 12 months of initiating therapy for metastasis.

**Results:** Overall, 14/28 (50%) tumors were FGFR4+, of those 12/14 (85.7%) were also resistant to trastuzumab. Of the entire cohort, 18/28 (64.3%) tumors showed resistance to trastuzumab, and of those, 12/18 (66.7%) expressed FGFR4 on IHC (Table 1). In invasive mammary carcinoma, a significant association between expression of FGFR4 and resistance to trastuzumab therapy was identified (odds ratio=0.125; 95% CI, 0.02 - 0.7819). In cell lines, Western blot analysis of five HER2/neu-amplified cases showed concomitant FGFR4 amplification in 4/5 (80%) cell lines. Of those, 2/4 (50%) demonstrated resistance to trastuzumab. Of interest, the 2 resistant cell lines were the ones with the highest level of FGFR4 amplification.

Table 1: Correlation of FGFR4 expression and response to trastuzumab in invasive breast carcinoma

| | FGFR4-positive | FGFR4-negative | p-value |
|---|---|---|---|
| Trastuzumab-resistant | 12 | 6 | 0.0461 |
| Trastuzumab-sensitive | 2 | 8 | |

*Note: FGFR4-positivity was defined as presence of at least moderate cytoplasmic and/or membranous expression in ≥10% of invasive carcinoma cells on IHC

**Conclusions:** Our results imply a possible relationship between FGFR4 upregulation and therapeutic resistance to trastuzumab in HER2-positive invasive mammary carcinomas in our cohort, suggesting the two genes may co-signal in breast cancer. Importantly, FGFR4 amplification may potentially assist in guiding patient selection for combination therapy, including FGFR4 inhibitor and anti-HER2 therapy.

## 185 Characteristics of HER2 FISH-Equivocal Breast Cancers and the Impacts on HER2 Status of 2018 ASCO/CAP Guideline

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[1]*Fudan University Shanghai Cancer Center, Shanghai, China*

**Disclosures:** Hui Kong: None; Qianming Bai: None; Hongfen Lu: None; Xiaoyan Zhou: None; Wentao Yang: None

**Background:** ASCO/CAP guideline on HER2 testing for breast cancers was updated in 2018. According to the new guideline, *HER2* FISH-equivocal breast cancers will be categorized as HER2 negative except those with HER2 IHC 3+. However, whether or not *HER2* FISH-equivocal breast cancers was a heterogeneous group due to different *HER2* copy number has not been well illustrated.

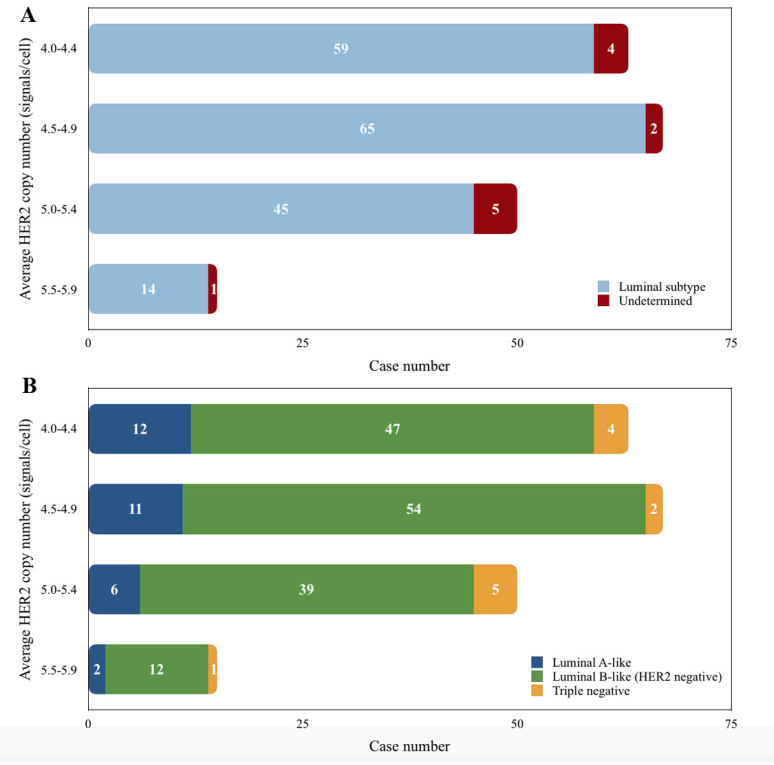
**Design:** According to 2013 ASCO/CAP guideline, 195 *HER2* FISH-equivocal invasive breast cancers were collected between 2014 and 2018, and IHC test was performed on same tissue used for FISH in all cases. The molecular subtype was determined according to 2013 St Gallen consensus, and HER2 status was also redefined following 2018 ASCO/CAP guideline. All cases were classified into 4 groups according to the average *HER2* copy number (4.0-4.4, 4.5-4.9, 5.0-5.4, 5.5-5.9 signals/cell). The relationship between *HER2* copy number and clinicopathological parameters was analyzed. Disease-free survival (DFS) and overall survival (OS) were estimated with Kaplan-Meier analysis.

**Results:** According to 2013 ASCO/CAP guideline, 183 (93.8%) of 195 FISH-equivocal cases were of luminal subtype, while the other 12 (6.2%) were undetermined. Using 2018 ASCO/CAP guideline, all FISH-equivocal cases were recategorized to HER2 negative. Therefore, 31(15.9%) cases were luminal A-like, 152 (77.9%) were luminal B-like (HER2 negative) and 12 (6.2%) were triple negative. The average *HER2* copy number showed positive correlation with polysomy 17, but had no significant association with other clinicopathological parameters. Survival analysis indicated that lymph node and distant metastases were predictive factors for DFS and OS, while *HER2* copy number had no significant impact on prognosis. 17 (8.7%) cases were treated with trastuzumab, but showed no difference in DFS or OS with patients who didn't receive targeted therapy.

| Characteristics | Average *HER2* copy number | | | | *P* value |
|---|---|---|---|---|---|
| | 4.0-4.4 | 4.5-4.9 | 5.0-5.4 | 5.5-5.9 | |
| **Age (year)** | | | | | 0.15 |
| **≤ 53** | 32 (50.8) | 41 (61.2) | 20 (40.0) | 5 (33.3) | |
| **＞53** | 31 (49.2) | 26 (38.8) | 30 (60.0) | 10 (66.7) | |
| **Histopathology** | | | | | 0.969 |
| **IDC*** | 58 (92.1) | 62 (92.5) | 49 (98.0) | 12 (80.0) | |
| **Non-IDC** | 5 (7.9) | 5 (7.5) | 1 (2.0) | 3 (20.0) | |
| **Histologic grade** | | | | | 0.129 |
| **I** | 0 (0.0) | 0 (0.0) | 2 (4.0) | 0 (0.0) | |
| **II** | 27 (42.9) | 34 (50.7) | 23 (46.0) | 10 (66.7) | |
| **III** | 36 (57.1) | 33 (49.3) | 25 (50.0) | 5 (33.3) | |
| **ER** | | | | | 0.538 |
| **Positive** | 59 (93.7) | 65 (97.0) | 45 (90.0) | 14 (93.3) | |
| **Negative** | 4 (6.3) | 2 (3.0) | 5 (10.0) | 1 (6.7) | |
| **PR** | | | | | 0.257 |
| **Positive** | 48 (76.2) | 57 (85.1) | 43 (86.0) | 12 (80.0) | |
| **Negative** | 15 (23.8) | 10 (14.9) | 7 (14.0) | 3 (20.0) | |
| **Ki-67** | | | | | 0.065 |
| **＜ 20%** | 19 (30.2) | 20 (29.9) | 7 (14.0) | 3 (20.0) | |
| **≥ 20%** | 44 (69.8) | 47 (70.1) | 43 (86.0) | 12 (80.0) | |
| **Polysomy 17** | | | | | ＜ 0.001** |
| **Yes** | 30 (47.6) | 48 (71.6) | 46 (92.0) | 13 (86.7) | |
| **No** | 33 (52.4) | 19 (28.4) | 4 (8.0) | 2 (13.3) | |
| **Primary Tumor (T)** | | | | | 0.776 |
| **T1** | 31 (49.2) | 33 (49.3) | 21 (42.0) | 8 (53.3) | |
| **T2** | 30 (47.6) | 33 (49.3) | 28 (56.0) | 7 (46.7) | |
| **T3** | 0 (0.0) | 1 (1.5) | 0 (0.0) | 0 (0.0) | |
| **T4** | 2 (3.2) | 0 (0.0) | 1 (2.0) | 0 (0.0) | |
| **Regional Lymph Nodes (N)** | | | | | 0.806 |
| **N0** | 32 (50.8) | 40 (59.7) | 25 (50.0) | 7 (46.7) | |
| **N1** | 20 (31.7) | 15 (22.4) | 17 (34.0) | 5 (33.3) | |
| **N2** | 8 (12.7) | 6 (9.0) | 3 (6.0) | 2 (13.3) | |
| **N3** | 3 (4.8) | 6 (9.0) | 5 (10.0) | 1 (6.7) | |
| **Distant Metastasis (M)** | | | | | 0.804 |
| **M0** | 62 (98.4) | 66 (98.5) | 50 (100.0) | 14 (93.3) | |
| **M1** | 1 (1.6) | 1 (1.5) | 0 (0.0) | 1 (6.7) | |

***: Invasive ductal carcinoma**

Figure 1 - 185




**Molecular subtype distribution of HER2 FISH-equivocal breast cancers in terms of average HER2 copy number according to 2013 (A) and 2018 (B) ASCO/CAP guideline**

**Conclusions:** In this study, all *HER2* FISH-equivocal breast cancers were reclassified to be HER2 negative according to 2018 ASCO/CAP guideline. Most of these patients were luminal B-like (HER2 negative). The average *HER2* copy number had no significant association with other clinicopathological parameters, as well as prognosis. Besides, DFS and OS showed no difference between patients with and without targeted therapy. Further underlying molecular mechanism study with long term follow-up is necessary for this special group of patients.

## 186 Benign Vascular Lesions and Angiolipomas of the Breast: Radiological-Pathological Correlation

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**Disclosures:** Oleksandr Kravtsov: None; Kurt Techawatanaset: None; Solomon Cherian: None; Julie Jorns: None

**Background:** Benign vascular lesions of the breast are relatively uncommon, frequently small and under-reported, but can mimic breast cancer clinically and/or on imaging. Smaller lesions are being detected with the recent progress in breast imaging modalities, such as MRI, however there are very few recent data available on radiological-pathological correlation on vascular lesions.

**Design:** A database search was performed for all angiolipomas, hemangiomas, intravascular papillary endothelial hyperplasia (IPEH) or Masson's tumors, and other benign vascular tumors of the breast from September 2006 to September 2018. Lesions on the breast skin (N=13) and atypical vascular lesions (N=3) were excluded. Overall 52 cases from 50 patients were found (22 hemangiomas, 29 angiolipomas, and 1 IPEH). Pathology slides (50 cases) and breast imaging (40 cases) were reviewed. All lesions were characterized via slide and imaging review.

**Results:** Most hemangiomas were incidental findings on pathology slides, but most of angiolipomas were primary biopsy targets (Table 1).

Radiologic-pathologic correlation was adequate in 30 of 40 cases with both slides and breast imaging available. Angiolipomas showed very good correlation, except 1 case that was only 0.05 cm. There was no radiologic correlate for 45% of hemangiomas, however median lesion size in those cases was only 0.25 cm, compared to 0.5 cm in cases with adequate correlation. Among the 10 cases that did not have radiologic correlate 6 cases had calcifications detected by mammography and not confirmed on H&E, 3 cases were seen as non-mass enhancement on MRI with no size provided, and 1 case had a 2.7 cm size discrepancy on imaging and H&E. MRI was performed in 4 cases in which there was no correlation and was not performed in the rest of the cases. Median size difference between radiologic and H&E measurements was 0.3 cm (range 0.1-0.9).

Angiolipomas (see Figures 1 and 2) had variability in composition, ranging from 5 to 95% vascular component (median 30%). Those with higher vascular composition (≥50%) were all diagnosed by core biopsy and were smaller (median 0.5 cm) as compared to those with more adipose content (18.8% diagnosed on core biopsy; median size 1.5 cm).

| | | | Rad-path correlation (40 cases: 20 H, 19 AL, 1 IPEH) | | | | |
|---|---|---|---|---|---|---|---|
| **Diagnosis** | **N (%)** | **Targeted lesion vs. incidental, N (%)** | **Adequate rad-path correlation** | | **No correlation** | | **Median rad-path size delta, cm** |
| | | | **N (%)** | **Median size, cm** | **N (%)** | **Median size, cm** | |
| **Hemangioma (H)** | 22 (42) | 8 | 11 | 0.5 | 9 | 0.25 | 0.2 |
| **Angiolipoma (AL)** | 29 (56) | 18 | 18 | 0.65 | 1 | 0.05 | 0.4 |
| **IPEH** | 1 (2) | 1 | 1 | 0.3 | | | 0.2 |
| **Total** | 52 | 27 (52) | 30 (75) | 0.5 | 10 (25) | 0.2 | 0.3 |

Figure 1 – 186

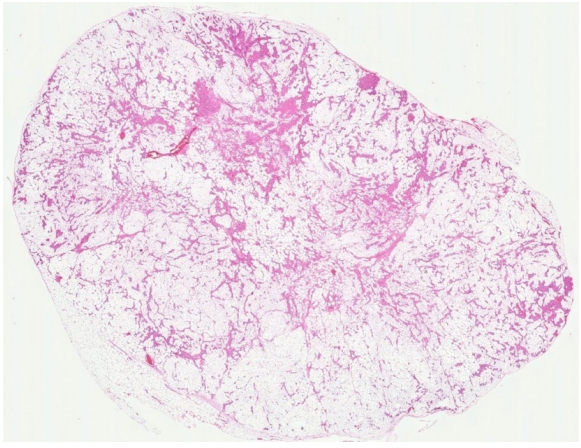
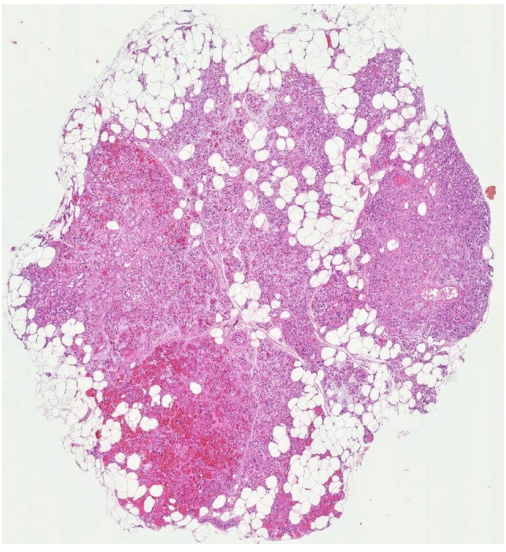


Figure 2 – 186



**Conclusions:** Benign vascular lesions of the breast have overall good radiologic-pathologic correlation with only small differences in size measurements. However, smaller lesions still provide correlation difficulties.

## 187 Genetics of Flat Epithelial Atypia and Early Neoplastic Progression in the Breast

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**Disclosures:** Gregor Krings: None; Eliah Shamir: None; Yunn-Yi Chen: None

**Background:** Morphologic, immunohistochemical, and molecular studies support a low grade neoplasia pathway in the breast (LGNP), which includes flat epithelial atypia (FEA), lobular neoplasia (LN), atypical ductal hyperplasia/low grade ductal carcinoma in situ (DCIS), and low grade invasive ductal (IDC), tubular (TC) and lobular carcinoma. Chromosomal copy number (CN) analysis supports the relatedness of these lesions, but little is known about the genetics of FEA or progression to more advanced precursors or carcinoma. We analyzed synchronous LGNP lesions using next generation sequencing (NGS) to better understand early neoplastic progression in the breast.

**Design:** DNA was extracted from 15 FEA and synchronous paired low (8) and intermediate (7) grade ER+/PR+ DCIS, LCIS (1), low grade IDC (1), tubular carcinoma (2), and normal tissue (15). NGS was performed targeting exons of 479 cancer genes and 40 introns. Duplicate reads were removed computationally for allele frequency determination and CN calling. Single nucleotide variants, insertions/deletions and CN alterations (CNA) were evaluated.

**Results:** Recurrent pathogenic alterations in FEA included PI-3 kinase pathway (PI-3K) genes (11/15 cases; 10/15 *PIK3CA*, 2/15 *AKT1*), *NCOR1* (5/15), *CBFB* (4/15), *GATA3* (3/15), *RUNX1* (2/15), and *FOXA1* (2/15). Subclonal *PIK3CA* heterogeneity was seen in 3/15 FEA. Of 4 FEA without PI-3K mutations, 3 had unusual morphology. Pathogenic alterations were shared between FEA and paired DCIS (14/15), LCIS (1/1), IDC (1/1), and TC (2/2), indicating common clonality. In only 1/15, DCIS with micropapillary features was genetically distinct from paired FEA and LCIS, which were clonally related to one another. *ERBB2* hotspot mutation was shared between FEA and DCIS in 1/15, which was the only case to recur with invasive carcinoma. FEA to DCIS progression showed additional pathogenic mutations in 6/14 cases, including *GATA3* (4/6) and *SETD2* (2/6), and additional CNA in 6/14 cases (mean 2, range 1-4), including 22q loss in 3/6. DCIS to invasive carcinoma progression was associated only with inactivating *ARID1A* mutation (1/3). FEA to LCIS progression showed *CDH1* mutation.

**Conclusions:** Lesions of the LGNP are clonally related, and characteristic genetic features of luminal A tumors are already present in FEA. Most additional mutations and especially CNA are suggested to occur with progression to DCIS rather than stromal invasion. LGNP does not show defined stepwise genetic progression as seen in some other epithelial neoplasias.

## 188 Gene Fusions and Molecular Alterations in Breast Cancer

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**Disclosures:** Melissa Krystel-Whittemore: None; Travis Rice-Stitt: None; Dennis Sgroi: None; Valentina Nardi: None; John Iafrate: *Major Shareholder*, ArcherDx; *Consultant*, Pfizer; *Grant or Research Support*, Blueprint Medicines; *Grant or Research Support*, Sanofi; *Consultant*, DebioPharm; Elena Brachtel: None

**Background:** Understanding molecular alterations in breast cancer is important for prognosis and targeted treatments. Recent studies of gene fusions in estrogen receptor (ER) positive breast carcinomas have revealed gene rearrangements and their subsequent expression serve as oncogenic mechanisms in cases of advanced breast carcinomas. These patients have rapid progression and shorter overall survival compared to breast cancer patients without gene fusions. However, studies on gene fusions in breast cancer are few. We evaluated gene fusions in breast carcinomas using our current in-house Solid Fusion Assay v2 (SFAv2), including ER+ and ER- tumors.

**Design:** We evaluated all fusion assays performed on breast carcinoma specimens (n= 223) from 207 patients using SFAv2 since its launch in May 2016. This RNA-based assay uses Anchored Multiplex PCR (AMP) to enrich for the targets of interest, which are then sequenced on an Illumina MiSeq or Next-Seq instrument. Fusion transcripts are detected and annotated using a laboratory-developed algorithm. We further analyzed cases with gene fusions for mutational alterations using our in-house Illumina next generation sequencing (NGS) platform, SNAPSHOT. ER, PR, and HER2 immunohistochemistry (IHC) was performed in all primary breast carcinomas, and complemented by HER2 fluorescence in-situ hybridization (FISH) in 8 cases.

**Results:** 14 breast carcinoma specimens from 11 patients showed 14 distinct gene fusions. 7 fusions have not been previously reported. NGS SNAPSHOT showed 21 oncogenic mutations in 9/11 patients. The most frequent mutation was in *TP53* (n=5, 24%). Patient age at breast cancer diagnosis was 53 years on average (range 35-77), with a median interval since breast cancer diagnosis of 2.5 years. 1 patient died of disease. Most frequently, patients had grade 3/3 ER+HER2- breast cancers (n=6, 55%). 1 patient was ER-/HER2+ (9%), and 4 triple-negative (36%). The patient who died had a *BRAF/SCAPER* fusion as well as *RB1* and *TP53* mutations. [Table 1]

**Table 1.** Gene Fusions and Molecular Alterations by Case

| Study Number | Left Partner Gene Name | Exon Left Partner | Right Partner Gene Name | Exon Right Partner | Novel Fusion | SNAPSHOT Variants |
|---|---|---|---|---|---|---|
| 1 | CRTC1 | 1 | MAML2 | 2 | N | Not performed |
| 2 | TOR1AIP2 | 3 | NOTCH2 | 27 | N | MYC, TP53 |
| 3 | C6orf211 | 4 | ESR1 | 5 | Y | ARID1A, KIT, DDX3X, BRCA2, GNA11 |
| | CCDC170 | 2 | ESR1 | 5 | Y | |
| 4* | ETV6 | 5 | NTRK3 | 15 | N | None detected |
| 5 | IGSF3 | 1 | NOTCH2 | 28 | Y | STK11 |
| 6 | IMPG1 | 12 | ESR1 | 1 | Y | PIK3CA, TP53 |
| 7 | GRIK1 | 8 | TMPRSS2 | 2 | Y | PIK3CA, TP53 |
| 8* | SCAPER | 15 | BRAF | 11 | Y | RB1, TP53 |
| 9 | INTS10 | Intron 11 | ESR1 | 5 | Y | MYC, MDM2, FGFR1 |
| 10 | CCDC170 | 2 | ESR1 | 3 | N | TP53 |
| 11 | SEC16A | 4 | NOTCH1 | 27 | N | ERBB2, PTCH1, TP53 |

* = Multiple fusions identified

**Conclusions:** We identified 14 gene arrangements (7 novel fusions) in 11 patients from our cohort of 223 breast carcinoma specimens. 5 of the fusions identified involved *ESR1*, all in patients with ER+ disease. 1/5 patients with *ESR1* fusions had not yet received hormonal therapy, whereas 4/5 fusions were observed post-treatment. 9/11 patients (82%) had oncogenic mutations, most commonly in *TP53*. This study identified novel and previously observed fusions in a cohort of ER+ and ER- breast tumors.

## 189 Comparison of Interobserver Variability in the Pathologic Diagnosis of Papillary Tumors in Breast Resection Specimens with 2012 WHO Classification

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**Disclosures:** Youngmee Kwon: None; Chung-Yeul Kim: None; So Yeon Park: None; Ji Shin Lee: None; Hyang Im Lee: None; Ho-chang Lee: None; Sun-Young Jun: None; Minjung Jung: None; Chang Won Jung: None; Soo Youn Cho: None

**Background:** The differential diagnosis of papillary lesions is the most difficult and challenging in breast pathology, as they span the spectrum of benign, atypical, and malignant even within one tumor as well as possess overlapping morphology and diverse terminology leading to several different diagnostic classifications.

**Design:** To investigate the agreement on the diagnosis of papillary lesions in breast resection specimens, each one representative hematoxylin and eosin (H&E) section from sixty breast resection specimens was reviewed by twenty breast pathologists from different institutions. And then immunohistochemical staining (IHC) was performed for p63 and CK5 in the same representative sections of all cases,

which were also reviewed by the same pathologists. Sixty papillary tumors were classified by WHO classification, four-tier, three-tier, and two different two-tier classification systems.

**Results:** WHO classification increased interobserver variability in comparison with any other classifications, showing the lowest kappa coefficient of 0.22 and 0.38 in H&E and IHC sections, respectively. In all classifications except differentiating intraductal and invasive tumors (from 0.52 to 0.55), there was remarkable increase in interobserver agreement after the use of IHC, revealing that unweighted kappa rose from 0.22, 0.39, 0.46, 0.50 on H&E only to 0.38, 0.53, 0.55, 0.64 in addition to IHC with WHO, four-tier, three-tier, and two-tier (benign vs. malignant) classification systems, respectively. The agreement was increased when classified with more simple systems as expected, demonstrating the concordance was relatively high with two tier systems when differentiating intraductal and invasive papillary tumors ($\kappa$=0.52 for H&E, $\kappa$=0.55 for IHC) or distinguishing benign from malignant papillary tumors ($\kappa$=0.50 for H&E, $\kappa$=0.64 for IHC). The kappa coefficient was the highest when differentiating benign and malignant papillary tumors with IHC ($\kappa$=0.64).

**Conclusions:** The immunohistochemical staining for basal cytokeratins and myoepithelial markers is helpful in increasing the diagnostic agreement of papillary tumors in breast resection specimens. But the concordance in the pathologic diagnosis of papillary tumors in breast resection specimens with WHO classification is significantly lower than any other classifications. Therefore, more efforts supporting intensive consensus study should be made to improve the agreement in the diagnosis and categorization of papillary breast tumors with WHO classification.

## 190 Pathologic Reporting Practices for Breast Cancer Specimens after Neoadjuvant Chemotherapy- A Survey Of Academic Breast Pathologists

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**Disclosures:** Sonali Lanjewar: None; Susan Fineberg: None; Priyanka Patil: None

**Background:** Neoadjuvant chemotherapy (NAC) is increasingly being used to treat primary invasive breast carcinoma. Response to NAC is an important determinant of prognosis and helps direct clinical decisions regarding need for additional therapies; hence accurate quantification of residual disease, both in the lymph nodes and breast, are critically important. An international working group has published recommendations for standardization of pathologic reporting of post NAC specimens, particularly in the setting of clinical trials. Based on these recommendations, we sent a survey to breast pathologists in academic centers across the United States regarding current reporting practices .

**Design:** We sent a survey questionnaire to breast pathologists in academic centers which consisted of questions with yes/no answer. The pathologists were encouraged to add any comments. The questionnaire was as follows: Do you grade tumors after NAC? Do you routinely repeat hormone receptors, Her2/Neu results after NAC? If there are features of tumor regression/tumor bed at the margin but no actual tumor at the margin do you report this? Do you report number of nodes with fibrosis/ changes of regression? Do you report RCB score on your report or at least provide information on your report so clinicians can calculate RCB.

**Results:** We received response from 21 breast pathologists from 17 academic centers across the US which included NY, Texas , California, Connecticut , Massachusetts, Rhode Island ,Indiana , Florida , Wisconsin , Pennsylvania ,Michigan , and Georgia . The questionnaire, recommendations by international working group and responses is highlighted in the table 1. General comments from participants are summarized. General comments from participants: Cytologic atypia due to treatment effect must be addressed. Some reporting clinican driven. Absence of defined prognostic significance of reporting recommendation. Unaware of recommendation. Clarification of RCB guidelines.

| Questionnaires | Recommendation | Yes | No |
|---|---|---|---|
| Grading Post-NAC | Recommended | 17 | 4 |
| Routinely Repeat receptors Post-NAC | No Consensus | 14 | 7 |
| Routinely Report regression “tumor bed” at margin | Recommended | 12 | 9(sometimes 5 of 9) |
| Routinely report nodes with fibrosis/treatment effect | Recommended | 17 | 4 |
| Provide information for calculating RCB | Recommended | 17 | 4 |

**Conclusions:** Although recommendations for standardization of pathologic reporting of post NAC breast cancer specimens exist, academic breast pathology practices across the country show significant variability in the practice and reporting of post-NAC cases. It is important to investigate reasons for variability and possible barriers to standard practice. A CAP synoptic template designed specifically for post NAC specimens might remove this variability and achieve uniform reporting practices.

## 191 Reproducibility of the Sensitivity to Endocrine Therapy (SET) Assay for Stage II/III Breast Cancer Within and Between Pathology Laboratories

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W. Fraser Symmans: *Major Shareholder*, Delphi Diagnostics; *Advisory Board Member*, Merck; *Advisory Board Member*, Almac Diagnostics

**Background:** The sensitivity to endocrine therapy assay measures the expression of 18 genes related to estrogen and progesterone receptors ($SET_{ER/PR}$ index), 4 genes for receptors and proliferation *ESR1*, *PGR*, *ERBB2*, *AURKA* (RNA4), and 10 control genes. It employs a straightforward Quantigene Plex hybridization method (Thermo Fisher, Waltham, MA; Luminex, Austin, TX) without need for purification of RNA from unstained FFPE tissue sections on slides. The SET2,3 assay defines categories using cutpoints for $SET_{ER/PR}$ index that are adjusted for known prognostic measures of T-stage, N-stage, and phenotype (RNA4) for Stage II/III breast cancer that is hormone receptor-positive and HER2-negative.

**Design:** This blinded study used a balanced replicated design to assess inter- and intra-laboratory reproducibility of $SET_{ER/PR}$ index, *ESR1*, *PGR*, *ERBB2*, *AURKA*, and SET2,3 class in 60 HR+/HER2- breast cancers (Figure 1). Laboratories used an HE section to guide macrodissection, applied lysis buffer to the tumor section, and followed a standard operating procedure for SET assay. The gene measurements and indices were calculated centrally using pre-defined statistical R-scripts. Concordance correlation coefficient (CCC) was calculated from continuous measurements, Kappa statistic was calculated for categorical results, and a mixed effect model estimated contributions to bias (fixed effects) and variance (random effects) from the replicated design.

**Results:** We observed highly concordant intra-laboratory and inter-laboratory measurements of the multigene $SET_{ER/PR}$ index and the 4 single genes (Table 1). The lower concordance of AURKA in lab Y was driven by 3 outlier measurements. The mixed effects model showed minimal contribution from laboratories on variability of measurements of $SET_{ER/PR}$ index (Figure 1), and indicated a small but statistically significant bias between laboratories (M vs Y, p=0.02; M vs A, p=0.01) that was unlikely to be meaningful (Figure 2). There was excellent agreement of SET2,3 class, with Fleiss' kappa value 0.84 across the 3 laboratories and 2 replicates (Table 1).

Table 1. Intra-laboratory and Inter-laboratory concordance of $SET_{ER/PR}$index and single gene measurements and agreement in SET2,3 class high versus low.

| | Concordance (CCC) | | | | | Agreement (%) | Agreement (Kappa) |
|---|---|---|---|---|---|---|---|
| Intra-Lab | $SET_{ER/PR}$index | *ESR1* | *PGR* | *ERBB2* | *AURKA* | SET2,3 class | SET2,3 class |
| M | 0.99 | 0.99 | 0.97 | 0.98 | 0.99 | 95 | 0.86 |
| Y | 0.99 | 0.99 | 0.96 | 0.89 | 0.51 | 97 | 0.90 |
| A | 0.97 | 0.99 | 0.87 | 0.96 | 0.98 | 93 | 0.82 |
| Inter-Lab | | | | | | N/A | 0.84 overall |
| M vs Y | 0.99 | 0.99 | 0.97 | 0.97 | 0.82 | | 0.88 |
| Y vs A | 0.99 | 0.99 | 0.95 | 0.90 | 0.85 | | 0.83 |
| M vs A | 0.99 | 0.98 | 0.93 | 0.91 | 0.99 | | 0.82 |

Figure 1 – 191

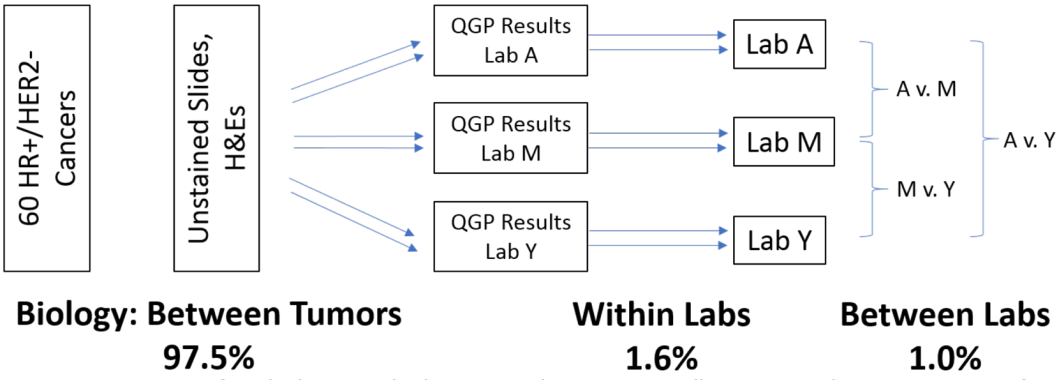


Figure 1. Summary of study design and relative contribution to overall variance in the measurement of $SET_{ER/PR}$ index measurements from FFPE tissue sections of 60 HR+/HER2- breast cancers.

Figure 2 - 191

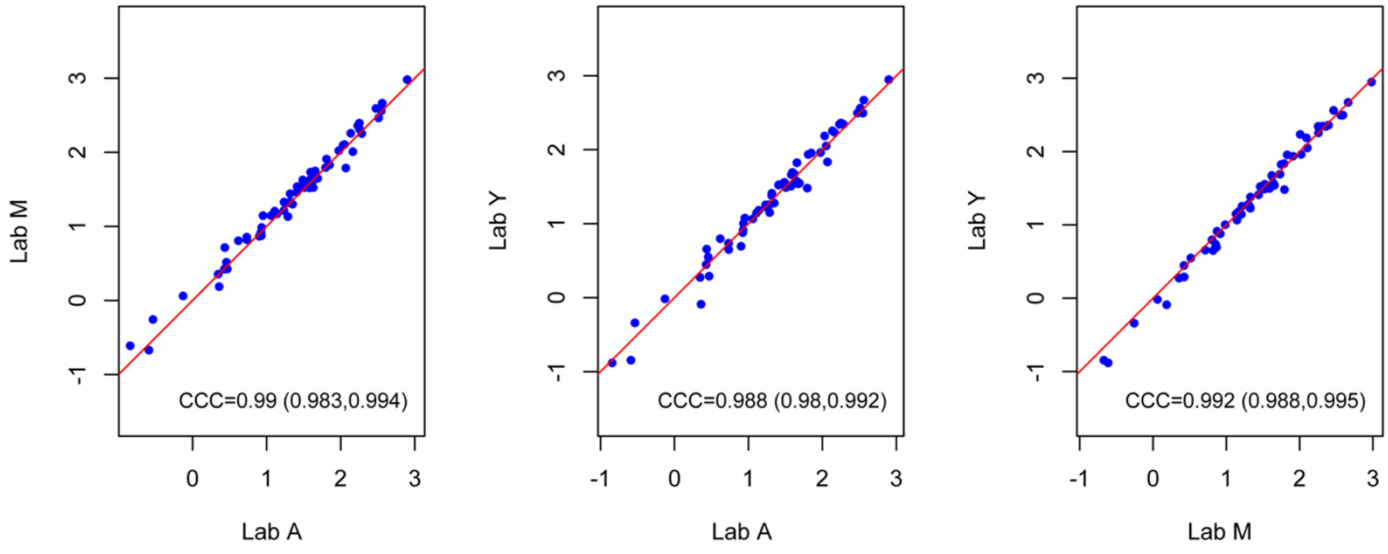


Figure 2. Scatterplots from inter-laboratory comparisons of SET ER/PR index (average of two replicates).

**Conclusions:** Estimated sensitivity to endocrine therapy from measurement of multigene $SET_{ER/PR}$ index and prognosis-adjusted assignment of SET2,3 class, and measurement of the individual RNA4 genes from FFPE tissue sections were highly reproducible within and between laboratories. Variance within and between laboratories was minimal compared to variance between cancer samples.

## 192 Quantitative Expression of MMPs 2, 9, 14, and Collagen IV in Lobular Carcinoma in Situ (LCIS) and Paired Normal Breast Tissue

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**Disclosures:** Thomas J. Lawton: None; Sarah Nyante: None; Tengteng Wang: None; Xianming Tan: None; Emily Ozdowski: None; Johann D. Hertel: None

**Background:** LCIS is considered a marker of increased breast cancer risk and recent literature suggests there may be subsets of LCIS which may also be pre-invasive lesions. One proposed mechanism of invasion in breast cancer is degradation of the basement membrane surrounding the in situ lesion. Matrix metalloproteinases have the ability to degrade collagen IV, a main component of the breast lobular basement membrane. Much of the existing data regarding MMP expression in LCIS and normal breast tissue comes from samples obtained from patients with co-existing invasive breast cancer. Given the known ability of tumors to influence host tissue, it is unclear whether MMP expression in these benign cases preceded the tumor, or if expression levels are a result of the tumor.

**Design:** In this cross-sectional study, we identified 55 women diagnosed with classic LCIS at a single academic institution between 2004 and 2014. Variants such as florid and pleomorphic LCIS were excluded. None of the patients had concomitant invasive carcinoma. 40 women also had sufficient normal breast tissue for analysis. We evaluated expression of matrix metalloproteinases (MMPs) 2, 9, and 14 and collagen IV in LCIS and adjacent normal breast tissue to determine whether these MMPs are expressed in benign tissue and LCIS. Marker expression was measured using immunofluorescence staining and quantified using the H score (MMPs) or pixel intensity (collagen IV). Expression associations were evaluated using the Spearman correlation. Differences between LCIS vs. normal expression were evaluated using the Wilcoxon signed-rank test.

**Results:** In LCIS and normal tissue, expression of MMP2 and MMP14 showed a strong correlation (LCIS $r=0.71$, normal $r=0.79$, both $P<0.01$). Other pairwise correlations were moderate (range, LCIS $r=0.33 – 0.53$, normal $r=0.20 – 0.33$). For all MMPs, expression was lower in LCIS when compared with normal tissue (all $P<0.05$). Collagen IV expression was also lower in LCIS compared with normal tissue ($P=0.05$).

**Conclusions:** MMPs associated with collagenase activity are expressed in the epithelium of normal breast tissue and in cases of LCIS in patients without associated invasive carcinoma. MMP expression was not higher in LCIS compared with normal tissue, suggesting that collagenase MMP expression may not increase as breast tissue gains a more proliferative phenotype.

## 193 Drugging tumour heterogeneity via targeting of Wnt signaling In breast cancer

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**Disclosures:** Victor Lee: None; Kee Wah Lee: None; Sai Mun Leong: None; Evelyn Siew-Chuan Koay: None; Muhammad Sufyan Bin Masroni: None; Karen Tan: None

**Background:** Therapy resistance and disease relapse remains a major challenge in medical oncology. Selective targeting of the dominant tumour clone has proven to be effective, but the observed responses are usually partial and not durable. Such observations have led to the suggestion that tumor behaviour may be determined not by its most aggressive parts, but rather, by a composite of the diversity within it. A potential therapeutic strategy will thus be to incorporate intra-tumor heterogeneity into treatment design. Through molecular profiling of circulating tumour cells (CTCs) harvested from breast cancer patients undergoing taxane-based chemotherapy, we have previously identified the up-regulation of miR-125b in a subset of breast cancer cell population that might contribute to taxane resistance. For this study, we seek to confirm the heterogeneous expression pattern of miR-125b using neoadjuvant taxane chemotherapy-treated breast tumour biopsies, as well as to determine the signaling pathways active in miR-125b-high vs. miR-125b-low breast cancer clones.

**Design:** MicroRNA *in situ* hybridisation was performed to visualize expression of miR-125b in 26 neoadjuvant taxane chemotherapy-treated and 27 treatment naïve breast tumour biopsies. Single cell RNA seq was performed for miR-125b-high vs. miR-125b-low isolated by sorting MDA-MB-231 which harbor a lentiviral construct driving EGFP expression under *hsa-miR-125b-1* promoter.

**Results:** We observed that significantly more breast tumour biopsies from patients with taxane-based neoadjuvant chemotherapy showed heightened miR-125b expression, as compared to patients without (chi square $= 20.6$, $p <0.0001$). Single-cell RNA sequencing reveals heightened expression of AGO2, AGO3 and FZD4 in the miR-125b-high cell population.

**Conclusions:** Non-canonical WNT pathway appears to be more active in miR-125b-high cells. Small molecule inhibitors targeting Wnt signalling pathway could be a potential combinatorial approach for disrupting miR-125b-associated clonal composition and augmenting the cytotoxicity of taxane agent.

## 194 Impact of Updated HER2 Guidelines on Immunohistochemical HER2-Equivocal Breast Carcinomas

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**Disclosures:** Eleanor Lewin: None; Chelsea Mehr: None; Laura Collins: None; Liza Quintana: None

**Background:** Hormone receptor (HR) and HER2 status are of critical clinical importance in newly-diagnosed breast carcinomas as they are both prognostic markers and predictive indicators of response to targeted therapies. HER2 may be assessed by immunohistochemistry (IHC) or in situ hybridization (ISH). HER2 IHC staining is scored from 0 to 3+, with scores of 0 and 1+ classified as negative, 3+ as positive, and 2+ as equivocal. Many institutions perform IHC initially and reflex to ISH testing in equivocal cases. At our institution, every newly diagnosed breast carcinoma case is tested by both IHC and FISH. The goal of this study was to evaluate the impact of the updated ASCO-CAP guidelines on the HER2 IHC equivocal (2+) cases.

**Design:** Data from cases of invasive breast carcinoma from 11/2016-6/2018 were collected. HER2 IHC and FISH copy number and ratios were recorded. We applied the updated guidelines to cases with non-classical FISH results (FISH groups 2-4, see table).

**Results:** A total of 742 cases were identified. 451 cases (60.8%) were classified as HER2 negative by IHC (0/1+). 75 cases (10.1%) were classified as HER2 positive by IHC (3+). 216 cases (29.1%) were classified as HER2 equivocal (2+) by IHC. Of the equivocal cases, 192 were classified as positive or negative by FISH: 28 positive (13%) and 164 negative (76%). The remaining 24 cases (11%) had non-classical FISH results. Per the 2013 guideline, alternative probes were used to determine HER2 status of group 4 (see table) cases; groups 2 and 3 were classified as positive. Of the 24 non-classical cases, 22 were initially classified as HER2 positive by 2013 guidelines and 2 were classified as HER2 negative. Applying the 2018 guideline to these 24 cases resulted in 3 positive and 21 negative cases, i.e. 19 cases (2.6% of the total) reclassified from positive to negative (see table).

| FISH Group | # of IHC 2+ Cases | HER2 2013 | | HER2 2018 | | HER2 Result Change |
|---|---|---|---|---|---|---|
| | | Positive | Negative | Positive | Negative | (% Total) |
| 2<br><br>(HER2/CEP17 ratio >= 2.0; HER2 copy number >= 4.0) | 3 | 3 | N/A | N/A | 3 | 3 (0.4) |
| 3<br><br>(HER2/CEP17 ratio < 2.0; HER2 copy number >= 6.0) | 3 | 3 | N/A | 3 | N/A | 0 |
| 4<br><br>(HER2/CEP17 ratio < 2.0; HER2 copy number 4.0-5.9) | 18 | 16 | 2 | N/A | 18 | 16 (2.2) |
| Total | 24 | 22 | 2 | 3 | 21 | 19 (2.6) |

**Conclusions:** In many institutions, HER2 IHC is utilized to triage equivocal (2+) cases for FISH. As expected, at our institution most cases were HER2 negative (60.8%) or positive (10.1%) by IHC. Of IHC 2+ cases (216, 29.1%), a majority were readily classified as FISH positive (28, 3.8%) or negative (164, 22.1%). Of the 24 cases requiring further evaluation, 19 would be reclassified from positive to negative, representing 2.6% of all HER2 cases evaluated. This is a small percentage; however, given that over 250,000 new breast cancers are diagnosed annually, this would translate to more than 6500 patients no longer offered HER2-targeted therapy each year.

## 195 Evaluation of M2 macrophages with multiplex immunohistochemistry in triple-negative breast carcinoma: the association with response to neoadjuvant chemotherapy

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**Disclosures:** Zaibo Li: None; Hiro Nitta: *Employee*, Roche Diagnostics; Peter Banks: *Consultant*, Ventana Medical Systems, Inc.; Anil Parwani: None

**Background:** Immune reaction with tumor infiltrating lymphocytes (TILs) has been extensively investigated in breast cancer. Programmed cell death 1 (PD-1) and its ligand (PD-L1) are key physiologic suppressors of cytotoxic immune reaction. There are two types of tumor associated macrophages: M1 macrophages (tumoricidal function) and M2 macrophage (pro-tumoral function). Our purpose was to investigate the relationship between M2 macrophages and response to neoadjuvant chemotherapy in triple-negative breast carcinoma (TNBC).

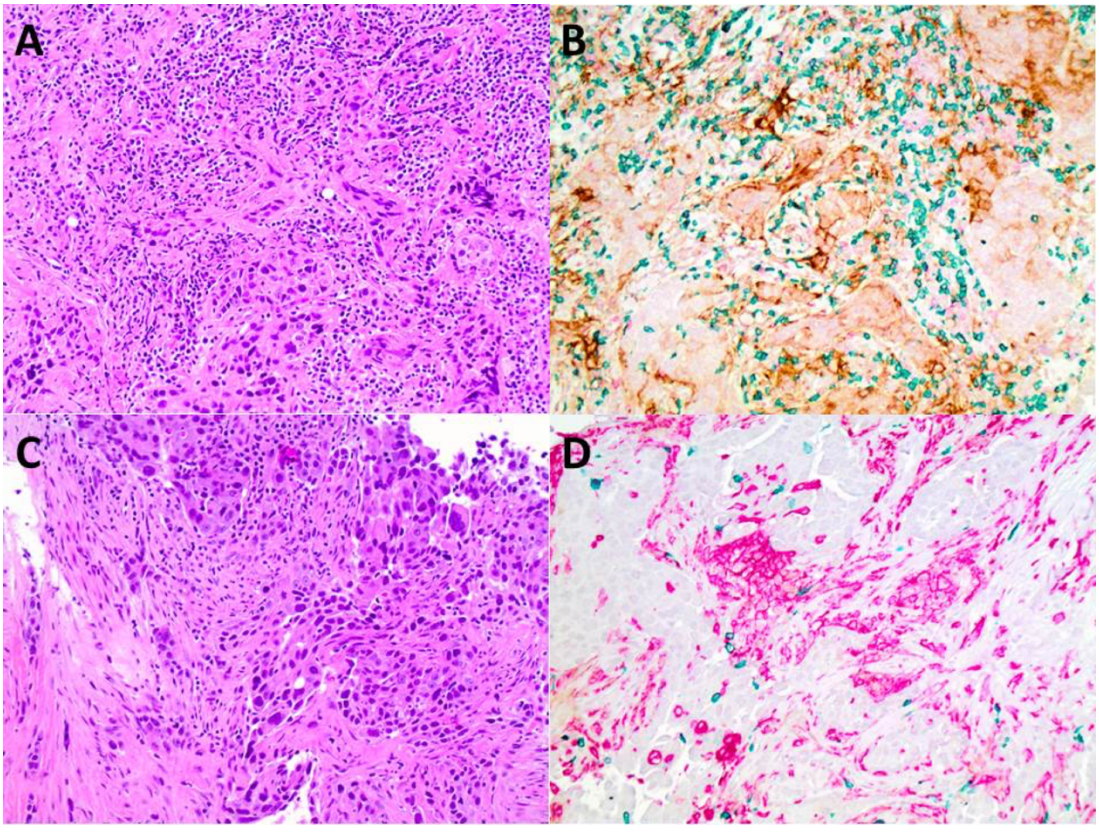
**Design:** A multi-color immunohistochemical multiplex assay simultaneously detecting PD1, PD-L1, CD8 (a marker for cytotoxic T cells) and CD163 (a marker specific for M2 macrophages) was performed on biopsy whole sections from 66 hormone receptor/Her2 triple-negative breast cancers treated with neoadjuvant chemotherapy (FIgure 1: One complete response case with high PD-L1, high CD8, but low CD163 (A, B) and one incomplete response case with no PD-L1, low CD8, but high CD163. A and C: H&E staining, 200x; B and D: multiplex immunostaining (PD-L1 in brown, CD8 in green and CD163 in red).

**Results:** By histologic examination of resection specimens following neoadjuvant therapy, 38 of the 66 cases had pathologic complete remission (pCR) and 28 had incomplete response. Compared to TNBCs with incomplete response, TNBCs with pCR were younger, had higher PD-L1 expression in tumor cells and stromal cells, higher levels of CD8-positive cells in stroma, and lower levels of CD163 expression in both tumor and stroma.

Table. The comparison of age, grade and checkpoint immune markers between triple-negative breast carcinomas with incomplete and complete response.

| | Incomplete response | | Complete response | | Total Cases | | p value |
|---|---|---|---|---|---|---|---|
| cases # | 38 | | 28 | | 66 | | |
| | Average | range | Average | range | Average | range | |
| Age (years) | 53.2 | 31-74 | 47 | 26-73 | 50.5 | 26-74 | 0.022 |
| Nottingham grade | 2.8 | 2-3 | 2.9 | 2-3 | 2.85 | 2-3 | 0.396 |
| PD1 (%) | 0.1 | 0-3 | 0.1 | 0-2 | 0.09 | 0-3 | 0.7996 |
| Tumoral PD-L1 (%) | 2.6 | 0-30 | 7.8 | 0-60 | 4.80 | 0-60 | 0.039 |
| Stromal PD-L1 (%) | 1.9 | 0-15 | 7.3 | 0-50 | 4.22 | 0-50 | 0.01 |
| Intratumroal CD8 (%) | 7.4 | 0-50 | 11 | 0-40 | 8.92 | 0-50 | 0.187 |
| Peritumoral CD8 (%) | 8.7 | 0-40 | 15 | 0-50 | 11.36 | 0-50 | 0.015 |
| Intratumoral CD163 (%) | 35.9 | 0-80 | 22.8 | 5-50 | 30.39 | 0-80 | 0.002 |
| Peritumoral CD163 (%) | 39.2 | 10-80 | 24.8 | 5-50 | 33.13 | 5-80 | 0.001 |

Figure 1 – 195



**Conclusions:** Our data confirm previous findings that pCR is associated with younger age, increased PD-L1 expression and CD8-positive lymphocytes. This represents the first demonstration of an association between pCR and a lower level of CD163 expression, suggesting that M2 macrophages play an important role in the host response to neoadjuvant chemotherapy.

## 196 Percentage of HER2 immunohistochemistry complete membrane staining is not significantly associated with response to neoadjuvant therapy in HER2 positive breast cancer

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**Disclosures:** Xiaoxian Li: None; Jing Zhao: None; Chao Zhang: None; Zaibo Li: None

**Background:** HER2 protein expression level is routinely evaluated by immunohistochemistry (IHC) in breast cancer. Breast cancer with HER2 IHC 3+ or HER2 gene amplification by FISH analysis is considered HER2 positive (HER2+). This study is to examine the correlation between HER2 protein expression level and response to neoadjuvant HER2 targeted therapy in HER2+ breast cancer.

**Design:** Seventy five consecutive HER2+ breast cancer biopsies were evaluated. All patients were treated with neoadjuvant HER2 targeted therapy. The response to neoadjuvant therapy was evaluated at the time of surgery. Cases with pathological complete response (pCR) or MD Anderson residual cancer burden I (RCB-1) were grouped as tumor with good response (responders). Cases with RCB-II and III were grouped as no response (non-responder). Percentage of HER2 IHC strong complete membrane staining in IHC 3+ cases and percentage of HER2 weak/moderate complete membrane staining in IHC 1-2+ (these cases had positive FISH result according to the 2018 ASCO/CAP recommendation) were correlated with response rate. We also correlated age, nuclear grade, Nottingham grade, tumor size, mitotic rate, ER, PR and Ki-67 with response rate.

**Results:** HER2 IHC 3+, high nuclear grade, young age, small tumor size and high Ki-67 were significantly associated with good response rate in multivariate analysis (all $P<0.05$). In the IHC 3+ group, 40 of 50 (80%) had good response while in the IHC1-2+ group, 8 of 25 (32%) had good response. In the IHC 3+ group, percentage of HER2 strong complete membrane staining was not associated with response rate. Similarly, the percentage of HER2 weak/moderate complete membrane staining was not associated with response rate in the HER2 IHC 1-2+ group.

**Conclusions:** HER2 IHC3+ was significantly associated with good response rate. Percentage of HER2 complete membrane staining was not associated with response rate in this cohort, supporting the current simple HER2 IHC classification.

## 197 Evaluation of tumor infiltrating lymphocyte, CD8+ and FOXP3+ cells and PD-L1 expression in tumor response to neoadjuvant therapy in HER2 positive breast cancer

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**Disclosures:** Xiaoxian Li: None; Jing Zhao: None; Yi Guo: None; Frank Schneider: None; Jane Meisel: None

**Background:** Tumor immune microenvironment is associated with prognosis and response to therapy in breast cancer. The role of tumor infiltrating lymphocyte (TIL), CD8+ and FOXP3+ lymphocytes and PD-L1 expression in tumor response to neoadjuvant therapy in HER2 positive (HER2+) breast cancer is not clear.

**Design:** We retrieved 85 HER2+ cancer biopsies diagnosed from 2010 to 2016. All patients were treated with neoadjuvant HER2-targeted and chemotherapy. Tumor response was evaluated at the time of surgery and classified as tumor with pathologic complete response (pCR, no invasive carcinoma in the breast and lymph nodes) and tumor without pCR. Forty six (54.1%) tumors had pCR and 39 (45.9%) did not achieve pCR. Age, tumor size before treatment, nuclear grade, Nottingham grade, ER, PR, Ki-67 and TIL of the carcinoma at biopsy were correlated with pCR rate. TIL was evaluated as average TIL and hot spot TIL (area of hot spot/area of entire intratumoral stroma). CD8 staining was evaluated in 20 biopsies with pCR and 27 without pCR. FOXP3 was evaluated in 18 biopsies with pCR and 26 without pCR. CD8+ and FOXP3+ cells were counted within 1mm around invasive carcinoma and the average absolute count of positive staining cells per high power field (HPF) was used to correlate with pCR rate. PD-L1 expression was evaluated in 20 biopsies with pCR and 27 without pCR.

**Results:** The absolute count of CD8+ cell was 60.1±40.8/HPF (average ± standard deviation) for tumors with pCR and 48.5±61.6/HPF for tumors without pCR. The absolute count of FOXP3+ cell was 32.2±14.6/HPF for tumors with pCR and 17.5±16.0/HPF for tumors without pCR. PD-L1 expression was detected in 4 tumors with pCR and in 1 tumor without pCR. FOXP3+ cells were significantly more prevalent in tumors with pCR ($P=0.014$) but the difference of CD8+ cell count was not significant. In univariate analysis, high FOXP3+ cell count,

high Ki-67 and high average TIL were significantly associated with pCR rate (all P<0.05). However, such significance disappeared in multivariate analysis. PD-L1 expression was not significantly associated with pCR rate.

**Conclusions:** FOXP3+ cells were significantly more prevalent in HER+ breast cancer with pCR. High FOXP3+ cell count and high TIL were associated with higher pCR rate in univariate analysis, indicating tumor microenvironment may play a role in response to therapy in HER2+ breast cancer. PD-L1 showed very low expression rate in HER2+ breast cancer and did not correlate with pCR rate in this study.

## 198 Impact of the updated 2018 ASCO/CAP guidelines for HER2 testing in breast cancer

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**Disclosures:** Anqi Li: None; Qianming Bai: None; Xiaoyan Zhou: None; Wentao Yang: None

**Background:** The 2018 ASCO/CAP guideline for HER2 testing in breast cancer consider both IHC and FISH results to define HER2 status in certain group of cases. Here we sought to evaluate its impact on designation of HER2 status in breast cancer.

**Design:** 2535 invasive breast cancers with HER2 IHC and FISH results diagnosed between 01/2014 and 08/2018 were included. The majority of patients with HER2 2+ tumors were suggested to have dual probe FISH tests. Clinical features, IHC and FISH results were retrieved from the pathology archives. According to HER2 value and HER2/CEP17 ratio, tumors were assigned into five groups.

**Results:** Hormone receptors were negative in 14% tumors, and were positive in 86% tumors. Ki-67 expression was <20% in 31% tumors, while ≥20% in 69% tumors. According to the updated guideline, 228 (9%) cases changed HER2 status, including 2.1% HER2+ tumors and 6.9% HER2 equivocal tumors both reassigned as HER2- (Table 1). In group 2, the mean count of HER2 was 3.4 (range 2.5-3.9), and of CEP17 was 1.5 (range 1.1-1.8). All tumors (2.1%) present with HER2 2+, previously considered as HER2+ by FISH, are now HER2-. Of 176 tumors (6.9%) in group 4, the mean count of HER2 was 4.7 (range 4-5.8), and of CEP17 was 3.2 (range 2.1-6.4). Of all the tumors in group 4, 6% tumors with HER2 0/1+ and 94% tumors with HER2 2+ changed HER2 status from equivocal into negative. All tumors (1%) in group 3 were with HER2 2+, thus remained the original FISH result as HER2+. Tumors in group 1 and 5 were with FISH HER2+ and HER2-, respectively. Therefore, 23% and 13% cases from group 2 changed from HER2+ luminal B into triple negative and luminal A subtypes, respectively. After reassigning HER2 equivocal as HER2-, group 4 now consists 5.7% triple negative, 21.6% luminal A and 72.7% luminal B subtypes.

Table 1. HER2 status of breast cancers in five groups.

| | Group 1 (n=333, 13%) | Group 2 (n=52, 2%) | Group 3 (n=27, 1%) | Group 4 (n=176, 7%) | Group 5 (n=1947, 77%) | % |
|---|---|---|---|---|---|---|
| HER2/CEP17 ratio | ≥2 | | <2 | | | |
| Average HER2 value (signals/cell) | ≥4 | <4 | ≥6 | 4≤ and <6 | <4 | |
| 2013 HER2 status | | | | | | |
| Positive | 333 | 52 | 27 | 0 | 0 | 16% |
| Negative | 0 | 0 | 0 | 0 | 1947 | 77% |
| Equivocal | 0 | 0 | 0 | 176 | 0 | 7% |
| HER2 IHC expression | | | | | | |
| 0/1+ | 7 | 0 | 0 | 11 | 213 | 9% |
| 2+ | 292 | 52 | 27 | 165 | 1729 | 89% |
| 3+ | 34 | 0 | 0 | 0 | 5 | 2% |
| 2018 HER2 status | | | | | | |
| Positive | 333 | 0 | 27 | 0 | 0 | 14% |
| Negative | 0 | 52 | 0 | 176 | 1947 | 86% |
| Equivocal | 0 | 0 | 0 | 0 | 0 | 0% |

**Conclusions:** Following the 2018 ASCO/CAP guideline, the proportion of HER2- tumors increases 9%, originating from tumors in group 2 with HER2 2+ and tumors in group 4 with HER2 equivocal. The practical implication of this diagnostic approach of HER2 status would require further validation.

## 199 MRI-Guided Core Needle Biopsy of the Breast: Low Yield for Malignancy in the Absence of Concurrent Breast Cancer

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**Disclosures:** Amy Lilly: None; Meredith Johnson: None; Cherie Kuzmiak: None; David Ollila: None; Johann D. Hertel: None; Siobhan O'Connor: None; Benjamin Calhoun: None

**Background:** Breast MRI may be used to screen women at high risk for breast cancer (BC) and to determine the extent of disease in patients with BC. The goal of this study was to determine the pathologic correlates of suspicious findings detected by breast MRI.

**Design:** A 3 year retrospective analysis (2014-2017) identified 79 women with 101 MRI-guided core needle biopsies (CNB). MRI studies and pathology slides were reviewed and correlated by breast radiologists and pathologists. The dominant (primary) histopathologic finding in the CNB and additional significant findings (secondary) were recorded.

**Results:** The mean age was 49.7 (range: 24-77) years. There were 57 (72%) patients with a history of prior (n=13) or concurrent (n=44) BC. The indication for MRI-guided CNB was to determine extent of disease in 46 patients (58%). Of the 101 MRI-detected lesions, 63 (63%) showed non-mass enhancement (NME) and 38 (38%) showed mass-type enhancement. The primary pathologic finding was benign in 78 (78%) followed by BC 17 (17%), and atypical hyperplasia (AH) 6 (5%). The most common benign findings were: fibrocystic changes (FCC) (41%), sclerosing lesions (10%), fibroadenoma (FA) (8%). All of the sclerosing lesions were associated with NME ($P$ = 0.0122) and all of the FA were associated with mass-type enhancement ($P$ = 0.0002). Of the 17 BC cases, 8 (47%) were invasive lobular carcinoma (ILC), 6 (35%) DCIS, and 3 (18%) were invasive ductal carcinoma (IDC). The BC detection rate was higher in CNB following MRI for extent of disease versus other indications (25% vs 4%; $P$ = .0129). Of the 15 BC detected in MRI-guided CNB following MRI for extent of disease, 12 were in the ipsilateral breast.

**Conclusions:** Most patients with suspicious breast MRI findings who underwent MRI-guided CNB had benign pathology. The most common benign and malignant diagnoses were FCC and ILC, respectively. Only 2% of the MRI-guided CNB in this series resulted in a new diagnosis of malignancy in patients without a prior diagnosis of BC.

## 200 Adenomyoepitheliomas of the Breast Frequently Harbor Recurrent Activating Hotspot Mutations in PI3K-AKT Pathway Related Genes

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**Disclosures:** Daniel Lubin: None; Erik Toorens: None; Shabnam Jaffer: None; Ezra Baraban: None; Paul Zhang: None; Ira Bleiweiss: None; Anupma Nayak: None

**Background:** Adenomyoepithelioma (AME) of the breast is a rare tumor morphologically defined by the presence of a biphasic population of ductal epithelial elements mixed with clear epithelioid to spindled myoepithelial cells. The genetic aberrations of AME have not been fully explored given its rarity. A recently published study utilizing massively parallel and whole exome sequencing found *PIK3CA* or *AKT1* activating mutations in a majority of AMEs as well as *HRAS* Q61 mutations in estrogen receptor (ER) negative AMEs (Geyer et al., Nature Communications, 9(1), 2018). We present here our findings on the molecular profile of AME.

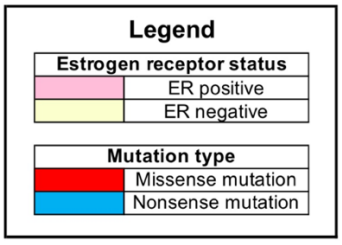
**Design:** Cases diagnosed as AME (1990 – 2018) were retrieved from pathology archives. H&E slides were reviewed by a Breast Pathologist to confirm the diagnosis. Next generation sequencing (NGS) covering 2,800 hotspot regions of 50 oncogenes and tumor suppressor genes was performed on 18 AME cases with adequate tumor tissue.

**Results:** Of 18 cases selected, 11 were classified as benign, 4 as atypical and 3 malignant. 15 cases were ER positive, and 3 ER negative. Two cases failed adequate DNA extraction. Of the remaining 16 cases, 13 had at least one nonsynonymous mutation identified as shown in Table 1 (allele frequency cut-off 5%). The most common recurrent mutations were *PIK3CA* p.H1047R (5/16) and *AKT1* p.E17K (5/16), which were found to be mutually exclusive. Of 6 tumors that did not have either an *AKT1* or *PIK3CA* mutation, 3 were wild type, 2 demonstrated mutations in *APC*, while 1 demonstrated an *STK11* pF354L mutation. Two cases with *AKT1* mutation had additional mutations in *ATM*, 1 case had additional *FGFR3* mutation and 1 case had additional mutations in *ATM, EGFR* and *GNAS*. One case with *PIK3CA* mutation had additional mutations in *IDH2, NOTCH1, RET* and *SMO* genes.

Figure 1 – 200

| | Benign | | | | | | | | | | | Atypical | | | | Malignant | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | #1 | #2 | #3 | #4 | #9 | #10 | #12 | #13 | #14 | #15 | #18 | #6 | #8 | #11 | #17 | #5 | #16 | #7 |
| AKT1 p.E17K | 35% | | | 21% | | | 42% | | | | | | 11% | | | 43% | | |
| PIK3CA p.H1047R | | 31% | 29% | | 43% | 26% | | | | | | | | 23% | | | | |
| PIK3CA p.E542K | | | | | | 7% | | | | | | | | | | | | |
| APC p.P870S | | | | | | | | | | | | 49% | | | | | | |
| APC p.A1564P | | | | | | | | | | | | 10% | | | | | | |
| APC p.E1317Q | | | | | | | | | | | | | | | | | | 48% |
| ATM p.V410A | 52% | | | | | | | | | | | | | | | | | |
| ATM p.F858L | | | | | | | | | | | | | | | | 47% | | |
| ATM p.P604S | | | | | | | | | | | | | 50% | | | | | |
| STK11 p.F354L | | | | | | | | 49% | | | | | | | | | | |
| EGFR p.E237K | 51% | | | | | | | | | | | | | | | | | |
| FGFR3 p.F386L | | | | | | | 44% | | | | | | | | | | | |
| GNAS p.R187H | 39% | | | | | | | | | | | | | | | | | |
| IDH2 p.W34X | | | | | | | | | | | | | | 7% | | | | |
| IDH2 p.V95I | | | | | | | | | | | | | | 6% | | | | |
| NOTCH1 p.T1573M | | | | | | | | | | | | | | 5% | | | | |
| RET p.R635C | | | | | | | | | | | | | | 6% | | | | |
| SMO p.R629K | | | | | | | | | | | | | | 7% | | | | |

Table 1: Mutations identified in AME cases with percentage allele frequency. Sequencing failed in cases #18 and #17.




**Conclusions:** AME of the breast frequently harbor recurrent activating hotspot mutations in PI3K-AKT pathway related genes. *PI3K* and *AKT1* mutations were mutually exclusive. In addition to the somatic mutations in *PIK3CA* and *AKT1*, our study found mutations not previously identified in AME: *ATM, APC, STK11, GNAS, EGFR,* and *FGFR3*. *APC* mutations were seen in two cases without *PIK3CA or AKT1* mutations, one of these atypical AME and one malignant AME. Mutations in *ATM* occurred in the presence of *AKT1* mutations. Interestingly, none of our cases had mutations in *HRAS or TP53*, perhaps related to the low frequency of ER negative cases in our cohort.

## 201 Adenomyoepitheliomas of the Breast: Do They Share Genetic Alterations of Epithelial-Myoepithelial Carcinoma of Salivary Glands?

Daniel Lubin[1], Paul Zhang[2], Shabnam Jaffer[3], Erik Toorens[4], Ezra Baraban[4], Ira Bleiweiss[5], Anupma Nayak[6]
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**Disclosures:** Daniel Lubin: None; Paul Zhang: None; Shabnam Jaffer: None; Erik Toorens: None; Ezra Baraban: None; Ira Bleiweiss: None; Anupma Nayak: None

**Background:** Adenomyoepithelioma (AME) of the breast and epithelial-myoepithelial carcinoma (EMC) of salivary gland are morphologically similar tumors defined by a presence of a biphasic population of ductal epithelial elements mixed with epithelioid to spindled myoepithelial cells. Roughly a third of EMCs demonstrate *HRAS* Q61 mutations, and a recent study identified evidence of a preexisting pleomorphic adenoma in most EMCs either by morphology or by the presence of *PLAG1*or *HMGA2* alterations (Hallani et al., Am J Surg Pathol, 42(1), 2018). Additional mutated genes identified in EMC include *PIK3CA, TP53, FBXW7,* and *SMARCB1*. We sought to further explore whether AMEs might also share the genetic alterations seen in EMC.

**Design:** Nine cases of AME retrieved from pathology archives of Mount Sinai Hospital, New York and the Hospital of the University of Pennsylvania were tested for the presence of *PLAG1* and *HMGA2* translocations by fluorescence in situ hybridization (FISH) using dual color breakapart probes. Next generation sequencing (NGS) covering 2,800 hotspot regions of 50 oncogenes and tumor suppressor genes was also performed.

**Results:** Of 9 AME cases, 6 were classified as benign, 2 as atypical, and 1 as malignant. The results of NGS and FISH studies are demonstrated in Figure 1. Sequencing failed in 2 cases. Of the remaining 7 cases, 3 harbored activating hotspot mutations in *PIK3CA*, and 1 demonstrated a mutation in *AKT1* and *FGFR3*. FISH results were uninterpretable in 3 cases (including the 2 for which sequencing failed).

No *PLAG1* rearrangements were identified in the remaining 6 cases. *HMGA2*gene was intact in 5 of the 6 cases; however, one case without any somatic mutations by NGS demonstrated a breakapart signal (a single orange signal without a corresponding green signal in addition to fused signals) in >50% of tumor cells, suggesting rearrangement.

Figure 1 - 201

| | Benign | | | | | | Atypical | | Malignant |
|---|---|---|---|---|---|---|---|---|---|
| | Case 1 | Case 2 | Case 3 | Case 4 | Case 5 | Case 6 | Case 7 | Case 8 | Case 9 |
| *PLAG1* status | Intact | Intact | Intact | Intact | Intact | | | | Intact |
| *HMGA2* status | Intact | Intact | Intact | Intact | Rearranged | | | | Intact |
| AKT1 p.E17K | | | 42% | | | | | | |
| PIK3CA p.H1047R | 43% | 26% | | | | | 23% | | |
| PIK3CA p.E542K | | 7% | | | | | | | |
| APC p.P870S | | | | | | | | | |
| APC p.A1564P | | | | | | | | | |
| APC p.E1317Q | | | | | | | | | |
| ATM p.V410A | | | | | | | | | |
| ATM p.F858L | | | | | | | | | |
| ATM p.P604S | | | | | | | | | |
| STK11 p.F354L | | | | | | | | | |
| EGFR p.E237K | | | | | | | | | |
| FGFR3 p.F386L | | | 44% | | | | | | |
| GNAS p.R187H | | | | | | | | | |
| IDH2 p.W34X | | | | | | | 7% | | |
| IDH2 p.V95I | | | | | | | 6% | | |
| NOTCH1 p.T1573M | | | | | | | 5% | | |
| RET p.R635C | | | | | | | 6% | | |
| SMO p.R629K | | | | | | | 7% | | |

**Legend**

| Mutation type |
|---|
| Missense mutation |
| Nonsense mutation |
| Failed |

Table 1: FISH and mutation status (with allele frequency) for 9 AME cases.

**Conclusions:** Among evaluable AME cases, a majority showed a mutation in either *PIK3CA* or *AKT1*. A single case without a mutation identified in NGS demonstrated a rearrangement of *HMGA2*. None of the cases harbored *HRAS* or *TP53*mutations. While no *HRAS* mutations or *PLAG1* rearrangements were identified, a single case revealed a rearrangement of *HMGA2*, and a few cases demonstrated *PIK3CA* mutations, which have been identified in EMC. Additional larger studies will be needed to further address the molecular relationship between AME and EMC.

## 202 Localized Amyloidosis: A Diagnostic Pitfall in Breast Biopsies

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**Disclosures:** Andrew Lytle: None; Farbod Darvishian: None; Ugur Ozerdem: None

**Background:** Amyloidosis is the extracellular deposition of insoluble protein fibrils in a β-pleated sheet configuration. While common systemic forms of amyloidosis show multiorgan involvement, amyloid deposition may instead be localized. Amyloidosis of the breast is a rare entity which has been reported to form solid masses with calcification. Breast amyloidosis has been observed in conjunction with systemic amyloidosis and predisposing diseases including plasma cell dyscrasias, lymphomas, and chronic inflammatory diseases. Details of the clinicopathologic features of localized breast amyloidosis remain limited.

**Design:** A retrospective search for breast amyloidosis diagnosed at our institution between 2005 and 2018 was performed and clinicopathologic features were documented.

**Results:** Nine patients with breast amyloidosis were identified. Patients were all female, with a mean age of 70 (range: 51-84), and lacked histories of amyloidosis prior to core biopsy diagnosis. Indications for breast or axilla biopsy were mammographic calcifications (56%) or mass (44%). Axillary lymphadenopathy was present in 2 (22%) patients.

Amyloid involvement was unilateral with left side predominance (78%) and showed positive staining with Congo Red in all cases. Amyloid type was determined in 6 cases using mass spectrometry or immunohistochemistry, revealing amyloid light chain (AL) λ in 3 cases, amyloid transthyretin (ATTR) in 2, and AL κ in 1 case. Four (44%) patients had histories of systemic autoimmune disease, which included systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, and polymyalgia rheumatica. Two (22%) patients had histories of lymphoid malignancy, including a previously treated marginal zone lymphoma and a new diagnosis of small B cell lymphoma of the breast. Four (44%) cases occurred alongside benign breast tissue, while 2 (22%) patients had concurrent breast neoplasia, including atypical ductal hyperplasia, lobular carcinoma in situ and ductal carcinoma in situ.

**Conclusions:** In our series, breast amyloidosis was of the AL or ATTR types, with a predominance of AL λ. In contrast to prior series, no patient had clinical evidence of systemic amyloidosis, although 66% of cases were associated with autoimmune disease or lymphoma. Localized amyloidosis should be considered in the differential diagnosis of all mammographic calcifications and masses of the breast or axilla, particularly in patients with a predisposing clinical history.

## 203 Residual Pure and Predominantly Pure Intralymphatic Breast Carcinoma Post Neoadjuvant Chemotherapy: Case Series and Next Generation Sequencing of a Rare Pattern of Disease

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**Disclosures:**Christine MacColl: None; Phillip Williams: None

**Background:** Locally advanced breast carcinoma is treated with neoadjuvant chemotherapy to shrink tumour size for optimal surgical results. The best case scenario is pathological complete response (pCR), which is associated with favourable long-term outcomes. The American Joint Committee on Cancer (AJCC) defines pCR as lack of invasive disease in the breast or lymph nodes, or only residual DCIS. There have been three studies which examined the unique pattern of pure or predominantly pure intralymphatic carcinoma in post-neoadjuvant mastectomies. These studies have been small, and the prognosis of this disease pattern remains unclear. The goals of the current study are to describe a series of patients with the rare finding of pure or predominantly pure intralymphatic breast carcinoma post-neoadjuvant chemotherapy and assess the genomic signature of this pattern of disease.

**Design:** Electronic medical records were searched to identify patients treated with post-neoadjuvant mastectomy between 2010-2017. Cases were included if they met the definition for pure or predominantly pure intralymphatic carcinoma, as defined by Rabban et al. Pure intralymphatic carcinoma was defined as residual disease only in lymphatic spaces. Predominantly pure was defined as intralymphatic carcinoma comprising at least 90% of the residual tumour. DNA was extracted from formalin-fixed paraffin embedded resection blocks containing intralymphatic carcinoma, nodal metastasis, and normal breast tissue. The genomic signature will be assessed using Next Generation Sequencing (NGS) and the Ion Ampliseq Cancer Hotspot Panel v2.

**Results:** Between 2010-2017, there were 479 post-neoadjuvant mastectomies performed at our institution. Upon review of all pathology reports and select mastectomy slides, it was determined that eleven patients had residual pure or predominantly pure intralymphatic carcinoma. Four patients (36%) died from metastatic disease within 25 months of mastectomy. Seven patients (64%) are alive. Additional pre- and post-neoadjuvant patient data is outlined in Table 1. NGS results are pending.

Table 1: Pre- and Post-Neoadjuvant Patient Data

| | |
|---|---|
| Age, *median* | 48 years |
| Pre-chemotherapy lesion size, *median* | 4.6 cm |
| Breast biopsy diagnosis | Invasive ductal carcinoma |
| Clinical stage | Locally advanced (stage III) |
| Chemotherapy | Doxorubicin, cyclophosphamide, paclitaxel (dose-dense AC/T) |
| Residual nodal stage, *N (%)*<br>ypN0<br>ypN1a<br>ypN2a<br>ypN3a | <br>4 (36%)<br>2(18%)<br>4 (36%)<br>1 (9%) |
| Post-operative follow-up, *median, range* | 34 months, 8-70 months |
| Outcome, *N (%)*<br>Died, metastatic disease<br>Alive, recurrence-free | <br>4 (36%)<br>7 (64%) |

Figure 1 – 203.

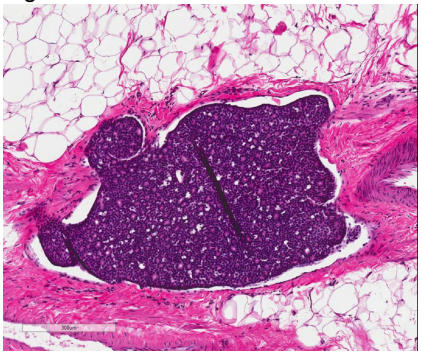
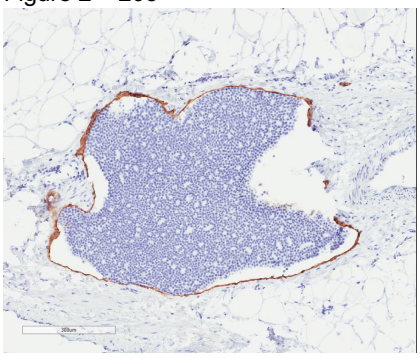


Figure 2 – 203



**Conclusions:** Pure and predominantly pure intralymphatic carcinoma in post-neoadjuvant mastectomies is extremely rare and this study contributes an additional eleven patients to the literature. Residual lymphovascular invasion should not be classified as pCR. Guidelines should be updated with recommendations on how to report and measure intralymphatic carcinoma in post-neoadjuvant mastectomies without residual invasive disease.

## 204 Papillary Lesions of the Breast: Diagnostic Findings that Support the Recommendation for Excision Following Core Biopsy

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**Disclosures:** Iris Martin: None; Laura Spruill: None

**Background:** The diagnosis of papillary lesion on core biopsy has routinely triggered the recommendation for excisional biopsy at our institution, given literature quoting a 10% upgrade rate. Recent single institution and small cohort publications cite a much lower rate of upgrade at excision, thus, our goal was to investigate our upgrade rate and attempt to risk stratify patients who could be spared additional surgery.

**Design:** All cases diagnosed as “Papillary Lesion” without a same-breast malignant or atypical finding from November 2012-November 2017 were identified, totaling 177 cases. 137 patients underwent excision and were included in the study. Age, race and presence or absence of atypia were noted, as was diagnosis at excision. Diagnostic upgrades at excision, including ADH, ALH, DCIS and invasive carcinoma were documented.

**Results:** Of the 137 total patients, 55 were Caucasian and 79 were African-American with the remainder other or unknown. Twenty-two percent of all cases were upgraded at excision. Of those nearly 12% were upgraded to carcinoma. Nineteen percent of all papillary lesions were diagnosed as “atypical” at core biopsy and 58% of upgrades derived from this group. Nine percent of non-atypical papillary lesions were upgraded at excision, but only 3% were upgraded to in situ or invasive carcinoma. While there was a statistically significant difference in patient age for diagnosis of atypia vs. non-atypical (62 y +/-13 vs. 54 y +/- 12, p=0.1), there was no difference in the age of patients without atypia who were upgraded at excision. No significant difference in the upgrade rate of white vs. black patients was observed.

**Conclusions:** These findings are largely consistent with published reports, but highlight the importance of noting atypia at the time of biopsy and in being attentive to the definition of an upgrade used in the literature. While only 3% of all non-atypical papillary lesions were upgraded to carcinoma at excision, 9% were upgraded to some level of atypia that would be of consequence for guiding patient follow up recommendations and/or chemoprophylaxis. These findings will help to inform patient decisions regarding excisional biopsy of non-atypical papillary lesions found at core biopsy.

## 205 HER2 testing in breast cancers: Comparison of Assays and Interpretation Using ASCO-CAP 2013 with 2018 guidelines

Lauren Mclemore[1], Constance Albarracin[1], Hui Chen[1], Roland Bassett[1], Sagar Dhamne[2], Stephen Gruschkus[1], Isaiah Yim[1], Kevin Lin[3], Yun Wu[4]
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**Disclosures:** Lauren Mclemore: None; Constance Albarracin: None; Hui Chen: None; Roland Bassett: None; Sagar Dhamne: None; Stephen Gruschkus: None; Isaiah Yim: None; Kevin Lin: None; Yun Wu: None

**Background:** HER2 amplification/overexpression is associated with aggressive clinical course, but predicts a better response to HER2 therapy. Immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) are used to evaluate HER2 overexpression and gene amplification, respectively. Oncotype (DX) used RT-PCR assay to measure 21 genes including HER2 (HER2 DX) to predict tumor recurrence in estrogen receptor(ER) positive(+), HER2 negative(-), node negative breast cancers. Despite established guidelines for HER2 testing, discordance has been reported among the different assays. In 2018 ASCO-CAP updated guidelines for HER2 reporting. The purpose of this study is compare concordance between HER2 DX, HER2 IHC and HER2 FISH using 2013 and 2018 ASCO-CAP guidelines at our institution.

**Design:** We retrospectively reviewed 657 patients with ER+, HER2- and node negative unifocal primary breast cancer with DX testing from May 2010 to May 2018. We reviewed the medical record and included histology, IHC and FISH results from our institution or outside institutions. The correlation of HER2 results between different assays and guidelines was examined using Kruskal's analysis.

**Results:** Of these 657 cases with DX, HER2 test were performed in 633 patients by IHC, 295 by FISH and 279 by both (Table 1). HER2 concordance between IHC and FISH was 213/279 (76.3%) by 2013 guideline and 214/279 (76.7%) by 2018 guideline, showing minimal change. One of 165 (0.6%) HER2 IHC- (1+) cases was considered as HER2+ using 2013 guideline and HER2- by 2018 guideline. This case is HER2- by DX. HER2 concordance between IHC and DX was 564/633 (89.1%). Of 630 HER2- by DX, 564 (89%) were negative by IHC and 66 (11%) equivocal (2+) by IHC. Three HER2 equivocal cases by DX were HER2- by IHC. HER2 concordance between FISH and DX was 279/295 (94.6%) using 2013 guidelines and increased to 294/295 (99.7%) by 2018 guidelines. All 294 HER2- by DX were interpreted as HER2- by FISH using 2018 guideline, however of these HER2- cases, 2013 guidelines interpret 15 cases as equivocal and 1 case as HER2+.

Table 1: Correlation of HER2 results between Oncotype DX versus IHC and FISH using ASCO/CAP 2013 and 2018 guidelines

| | Oncotype DX | | | | p-value | Concordance |
|---|---|---|---|---|---|---|
| | HER2- | | HER2 Equivocal | | | |
| **HER2 IHC** | 304 | 48.3% | 1 | 33.3% | 0.711 | 564/633, 89.1% |
| **Score 0 (N=305)** | 260 | 41.3% | 2 | 66.7% | | |
| **Score 1+ (N=262)** | 66 | 10.5% | 0 | 0% | | |
| **Score 2+ (N=66)** | 24 | - | 0 | - | | |
| **N/A (N=24)** | | | | | | |
| **HER2 FISH 2013** | 278 | 94.6% | 0 | 0% | 0.058 | 279/295, 94.6% |
| **Negative (N=278)** | 15 | 5.1% | 1 | 100% | | |
| **Equivocal (N=16)** | 1 | 0.3% | 0 | 0% | | |
| **Positive (N=1)** | 360 | - | 2 | - | | |
| **N/A (N=362)** | | | | | | |
| **HER2 FISH 2018** | 294 | 100% | 1 | 100% | - | 294/295, 99.7% |
| **Negative (N=295)** | 0 | 0% | 0 | 0% | | |
| **Positive (N=0)** | 360 | - | 2 | - | | |
| **N/A (N=362)** | | | | | | |

Figure 1 - 205

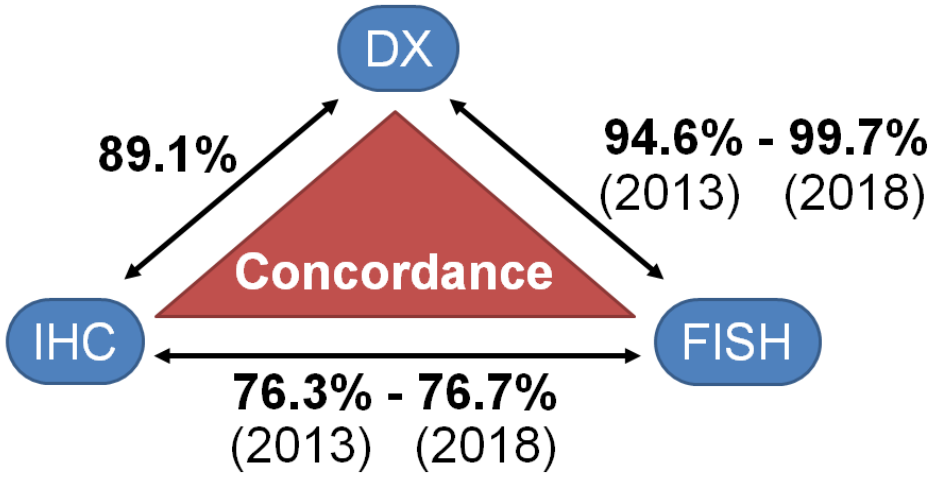


Figure 1: Concordance of HER2 testing by immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and reverse transcriptase polymerase chain reaction assay Oncotype DX (DX).

**Conclusions:** Our results demonstrate that both IHC and FISH assay have good concordance with HER2 DX. Concordance was better for FISH assay and marginally more so for 2018 guidelines (99.7%) compared to 2013 guidelines (94.6%). These results suggest that HER2 DX is not superior to existing FISH assay.

## 206 Estrogen receptor immunohistochemical staining versus Oncotype DX: Clinicopathologic characteristics and recurrence scores of concordant and discordant cases

Lauren Mclemore[1], Constance Albarracin[1], Hui Chen[1], Sagar Dhamne[2], Roland Bassett[1], Stephen Gruschkus[1], Isaiah Yim[1], Kevin Lin[3], Yun Wu[4]
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**Disclosures:** Lauren Mclemore: None; Constance Albarracin: None; Hui Chen: None; Sagar Dhamne: None; Roland Bassett: None; Stephen Gruschkus: None; Isaiah Yim: None; Kevin Lin: None; Yun Wu: None

**Background:** ER has been established prognostic and predictive biomarker in breast cancers and ASCO-CAP has defined guidelines for reporting them by immunohistochemistry (IHC). Oncotype DX (DX), a RT-PCR assay analyzing 21 genes including ER has the ability to predict recurrence risk in ER+ LN- breast cancers. Several studies have shown good concordance between ER IHC and DX, however a systematic correlation between IHC expression, recurrent scores, and histologic features is lacking. The aim of this study is to compare ER by IHC versus DX and correlate the findings with clinicopathologic features, Ki67 and recurrent score (RS) indexes

**Design:** We retrospectively reviewed 657 patients with ER+, HER2- and LN- unifocal primary breast cancer at our institution with DX testing from 2010 to 2018. We reviewed medical records and included histology and IHC results. Chi-squared/Fisher's exact tests evaluated associations between tests and concordance measured % agreement

**Results:** Of these 657 ER+ IHC cases, 629 were ER+ DX (96%) and 28 ER- DX (4%). The overall concordance between DX and IHC was 96%. The 28 ER DX discordant cases had ER IHC staining in <50% tumor cells in 16/28 (57%), had weak ER IHC staining intensity in 14/28 (50%) and moderate intensity in 13/28 (46%). Most of the ER DX discordant cases were also negative for PR in 25/28 (89%) with high Ki67 staining in 13/16 (81%), high nuclear grade in 25/28 (96%), high risk Genomic Health RS in 26/28 (93%). In contrast, the 629 ER+ DX concordant cases stained >50% tumor cells in 609/629 (97%) and ER IHC staining intensity was strong in 550/629 (87%). Most of the ER DX concordant cases were also positive for PR (536/629; 85%) and had low (341/629; 54%) or moderate (128/629; 20%) Ki67 staining. Nuclear grade was low in 17/629 (17%), intermediate in 417/629 (66%) and high in 104/629 (17%). Genomic Health RS was low risk in 327/629 (52%), intermediate risk in 246/629 (39%), and high risk in 56/629 (9%).

Figure 1 - 206

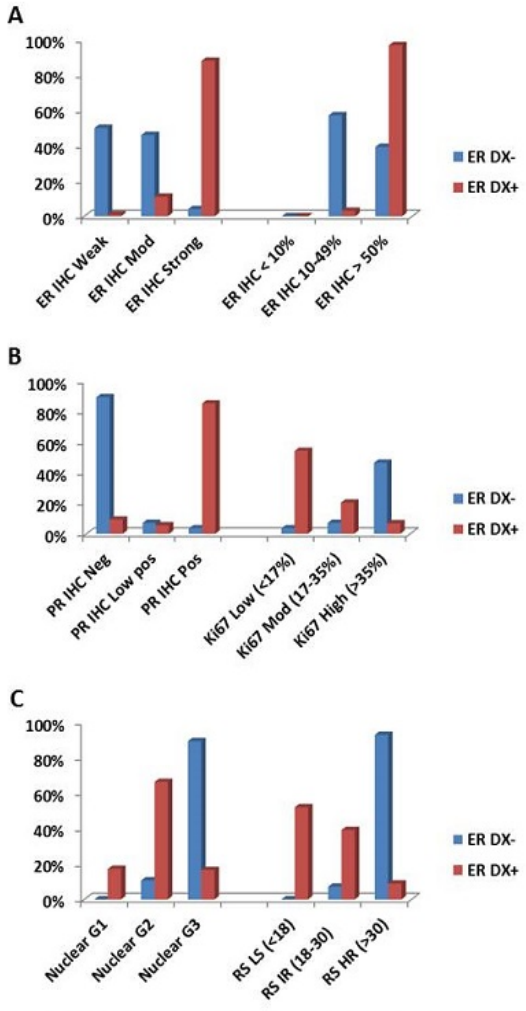


Figure 1: A) ER Oncotype DX versus ER IHC staining intensity and % tumor cells staining; B) ER Oncotype DX versus PR IHC and Ki67 IHC expression; C) ER Oncotype DX versus nuclear grade and Genomic Health Recurrence Score risk categories

**Conclusions:** Breast cancers with discordant ER DX results tend to have weak to moderate ER IHC staining, PR-, high Ki67, high nuclear grade and high risk RS. Breast cancer with concordant ER IHC and DX is associated with strong ER nuclear staining, PR+, low Ki67, intermediate nuclear grade, and low risk RS. Our results suggest that patients with weak to moderate ER staining may require integrated review of ER status with histological features and ancillary studies to guide treatment regimen.

## 207 Increased Expression of the Oncofetal Proteins IGF2BP2 and HMGA2 is Associated with Malignant Features in Phyllodes Tumors of the Breast

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**Disclosures:** Emily Mcmullen: None; Dafydd Thomas: None; Celina Kleer: None

**Background:** Phyllodes tumors (PT) are fibroepithelial neoplasms of the breast that are classified as either benign, borderline, or malignant based on several histopathologic criteria. The histologic diagnosis is often difficult, and may not predict clinical behavior. Furthermore, adequate classification of these tumors has become increasingly challenging, especially in small core-needle biopsies. IGF2BP2, and its pathway protein, HMGA2 are oncofetal proteins that are expressed during development, and are reduced in adult tissues. However, these proteins are seen to be re-expressed in malignant tumors, including liposarcoma and rhabdomyosarcoma,

and are thought to play a role in mesenchymal cell differentiation. We hypothesized that in PT, IGF2BP2 and HMGA2 expression may be associated with malignant features.

**Design:** Twenty-two PTs resected from 2006-2015 at our institution were histologically classified following WHO criteria, blinded to outcome, and used to develop a tissue microarray, in duplicate. Immunohistochemistry (IHC) for HMGA2, and IGF2BP2 were performed on TMA sections. Expression for IGF2BP2 and HMGA2 was assessed on a four-point scale (0, 1, 2, 3) and staining ≥1 was considered positive.

**Results:** Of the 22 PTs, 12 (54%) were benign, 7 (32%) borderline, and 3 (14%) malignant. Concordant upregulation of IGF2BP2 and HMGA2 in the stromal and/or epithelial cells was observed in 60% (9/15) of PT. Increased expression of IGF2BP2 and HMGA2 was significantly associated with the histological type of PT (Benign: 42% and 33%; Borderline: 57% and 57%; Malignant: 100% and 67%, respectively) (p=0.043). Mean staining intensity of IGF2BP2 increased significantly with increased severity of disease (0.58, 0.86, and 0.71, p=0.022). While a trend of increased mean intensity of HMGA2 staining was also seen with increased severity of disease (0.33, 0.71, 1.33), the differences were not statistically significant (p=0.1).

**Conclusions:** IGF2BP2 and HMGA2 showed concordant expression in a subset of PT, and showed increased expression and staining intensity in PT with malignant features. These findings may shed light into the pathogenesis of the malignant behavior of borderline and malignant PT and may be a useful diagnostic tool when working with difficult, small core-needle biopsies of PT.

## 208 Ductal Carcinoma In-Situ Exhibit Upregulation of Phosphorylated EZH2 (T367) in the Cytoplasm: Identification of Potentially Aggressive Biologic Behavior

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**Disclosures:** Emily Mcmullen: None; Stephanie Skala: None; Talha Anwar: None; Maria Gonzalez: None; Celina Kleer: None

**Background:** Ductal carcinoma in-situ (DCIS) is a non-invasive heterogeneous neoplastic growth and precursor of invasive carcinoma. There is limited understanding of the molecular alterations of DCIS, which may inform selection of markers to predict DCIS progression to invasive disease. In normal cells, the canonical function of EZH2 is to regulate cell differentiation by trimethylation histone H3 at lysine 27 trimethylation (H3K27me3). Our lab discovered a non-canonical function of EZH2 in aggressive triple-negative breast cancer: upon phosphorylation at threonine 367 (pEZH2 T367), a proportion of EZH2 protein localizes to the cytoplasm to promote H3K27me3 - independent increased invasion. However, the expression and localization of pEZH2 T367 in DCIS and its relationship to H3K27me3 are unknown. We hypothesize that a subset of DCIS may exhibit pEZH2 T367 in the cytoplasm and that this feature may signal biologically aggressive features.

**Design:** 124 invasive carcinomas and 35 DCIS cases resected from 1987-1992 at our institution were reviewed blindly by two pathologists and used to develop a tissue microarray, in duplicate. Immunohistochemistry (IHC) for pEZH2 T367 (using an antibody developed and validated in our lab) and H3K27me3 were performed on TMA sections. pEZH2 T367 and H3K27me3 were evaluated by intensity (four-point scale; 0-1 low, 2-3 high), localization (nuclear and/or cytoplasmic), and correlated with clinicopathologic features.

**Results:** Of the 124 invasive carcinomas, 102 (82%) were ductal, 7 (6%) lobular, and 15 (12%) other types. Of the 35 DCIS, 7 (20%) were low nuclear grade, 10 (29%) intermediate nuclear grade, and 18 (51%) high nuclear grade. 40% of the invasive carcinomas had high cyto-pEZH2/low nuclear-pEZH2 and 40% of DCIS showed low cyto-pEZH2/high nuclear-pEZH2 (p<0.001). In all cases, high nuclear-pEZH2 was associated with high H3K27me3, and high cyto-pEZH2 was associated with low H3K27me3. Nineteen of 35 (54%) DCIS cases showed high cyto-pEZH2 expression, of which 10/18 (55%) were >1 cm, 14/16 (88%) showed comedonecrosis, and 11/19 (58%) had high nuclear grade compared to low cyto-pEZH2.

**Conclusions:** We demonstrate that a subset of DCIS exhibit upregulation of EZH2 non-canonical pathway (cyto-pEZH2 T367). The expression of pEZH2 in the cytoplasm of DCIS may signal a higher propensity for progression, as they exhibit larger size, comedonecrosis, and high nuclear grade, which warrants further investigation.

## 209 Evaluating tumor mutation burden and its association with clinicopathologic variables and genetic alterations in breast cancers

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**Disclosures:** Ping Mei: None; C. Eric Freitag: None; Anil Parwani: None; Zaibo Li: None

**Background:** Immunotherapy has proven durable responses in a subset of cancer patients, and tumor mutation burden (TMB) has shown promise of being a biomarker to predict response in non-small cell lung cancer and melanoma. We aimed to investigate TMB in breast cancers and its association with clinicopathologic variables and genetic alterations.

**Design:** Sixty-three breast cancers (13 primary and 49 metastatic) were included and TMB was measured by hybrid based next generation sequencing (NGS) from Foundation Medicine, which has been shown to provide compatible results with whole exome sequencing (WES). TMB levels were divided into three groups based off the Foundation Medicine official reports: low (1-5 mutations/mb), intermediate (6-19 mutations/mb), and high (≥ 20 mutations/mb). Genetic alterations were also determined by Foundation Medicine NGS.

**Results:** Among 62 cases, 3 (4.8%) showed high TMB, 27 (43.6%) showed intermediate TMB, and 32 (51.6%) showed low TMB. Thirty-eight (61.3%) cases were invasive ductal carcinoma, 6 (9.7%) were invasive lobular carcinoma, 2 (3.2%) were metaplastic carcinoma, 16 (25.8%) were unknown. As for breast cancer biomarkers, 36 (58.1%) were ER positive, 38 (61.3%) were PR positive, 5 (8.1%) were HER2 positive, and 22 (35.5%) were triple negative. P53 mutations were the most common genomic mutation, detected in 37(59.7%) cases, followed by PI3KCA (33.9%, 21 cases). No significant difference was observed in age, ER, PR, HER2 status, histologic types between TMB high/intermediate and TMB low groups. TMB high/intermediate cases showed significantly more BRCA mutations (5/30=16.7%) than TMB low cases (1/32=3.1%) (p =0.00022), but not any other genomic alterations. (Table)

| | | Total | | TMB high/intermediate | | TMB low | | p value |
|---|---|---|---|---|---|---|---|---|
| Case # | | 62 | | 30 | | 32 | | |
| Age | | 53.8 | 30-78 | 54.6 | 31-74 | 53.1 | 30-78 | NS |
| Location | Primary | 13 | 21.0% | 6 | 20.0% | 7 | 21.9% | NS |
| | Metastatic | 49 | 79.0% | 24 | 80.0% | 25 | 78.1% | NS |
| Biomarkers | ER-positive | 36 | 58.1% | 19 | 63.3% | 17 | 53.1% | NS |
| | PR-positive | 18 | 29.0% | 9 | 30.0% | 9 | 28.1% | NS |
| | HER2-positive | 5 | 8.1% | 2 | 6.7% | 3 | 9.4% | NS |
| | Triple-negative | 22 | 35.5% | 8 | 26.7% | 14 | 43.8% | NS |
| Histologic type | Invasive ductal | 38 | 61.3% | 19 | 63.3% | 19 | 59.4% | NS |
| | Invasive lobular | 6 | 9.7% | 3 | 10.0% | 3 | 9.4% | NS |
| | Metaplastic | 2 | 3.2% | 1 | 3.3% | 1 | 3.1% | NS |
| | Unknown | 16 | 25.8% | 7 | 23.3% | 9 | 28.1% | NS |
| Genomic alterations | p53 | 37 | 59.7% | 20 | 66.7% | 17 | 53.1% | NS |
| | PI3KCA | 21 | 33.9% | 9 | 30.0% | 12 | 37.5% | NS |
| | BRCA | 6 | 9.7% | 5 | 16.7% | 1 | 3.1% | 0.0002 |

**Conclusions:** High TMB was observed in a relatively low percentage of breast cancers. The association between BRCA mutations and increased TMB is consistent with the importance of BRCA proteins in DNA repair function, suggesting breast cancer patients with BRCA mutation may benefit from immunotherapy.

## 210 Correlation of Hormone Receptor and HER2 Status Between RT-PCR and Immunohistochemistry in Invasive Breast Cancer

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**Disclosures:** Amanda Meindl: None; Edernst Noncent: None; Brian Finkelman: None; Tiansheng Shen: None; Luis Blanco: None; K. P. Siziopikou: None; Jennifer Pincus: None

**Background:** Estrogen receptor (ER), progesterone receptor (PR), and HER2 are important prognostic and predictive markers in breast cancer, routinely determined by immunohistochemistry (IHC) and/or fluorescence in situ hybridization (FISH) for HER2. The OncotypeDX™ Recurrence Score (ORS), uses RT-PCR to evaluate genes involved in cancer proliferation pathways, and is used as a prognostic indicator for response to chemotherapy and risk of recurrence. New guidelines for testing and interpretation of HER2 were recently released, however, there was insufficient data for molecular testing so it was not included in the update. We evaluated the results of ER, PR, and HER2 as reported by ORS and correlated this to the expression of these markers using the current ASCO-CAP approved IHC and/or FISH evaluation.

**Design:** The pathology database was searched for patients with invasive carcinoma and ORS testing from 2011–2014. Clinicopathologic characteristics, as well as ER, PR and HER2 IHC/FISH results were recorded according to ASCO-CAP guidelines. ORS hormone receptor status was reported as positive or negative. Concordance was assessed between IHC and RT-PCR status (+ or -) and quantified using Bangdiwala's B-statistic (accounts for agreement due to chance and ranges from 0-1, with 0=no agreement and 1=perfect agreement) where disagreement was present.

**Results:** One-hundred twenty patients were identified, ages 33-77 years old (mean = 56). In all cases, ER was positive and concordant by IHC and RT-PCR (100%). PR was concordant in 104/120 cases (87%) (B = 0.85; 95% CI 0.77, 0.92). In the discordant cases, 11 (70%) were IHC+/ RT-PCR-, and 5 (30%) were IHC-/ RT-PCR+. HER2 by IHC and/or FISH and RT-PCR was concordant in 119/120 cases (99%) (B = 0.99; 95% CI 0.98, 1.00). Of these, 114/119 (96%) were HER2-, and 4 cases were IHC score 2+ but FISH negative and RT-PCR- (3%). One case was discrepant for HER2 (IHC score 3+/ RT-PCR-). Twelve patients had recurrences during the 4-7 year follow-up period (10%). One case showed a discrepancy between PR in the initial tumor, (IHC+/ RT-PCR-) and was PR IHC+ in the recurrence.

**Conclusions:** RT-PCR can reliably predict hormone receptor and HER2 status, similar to IHC in invasive breast cancers, consistent with ASCO-CAP approved testing and reporting. Concordance was especially high for ER and HER2, markers critical to driving therapy in this population. As molecular testing becomes more common, ER, PR, and HER2 testing by RT-PCR should be considered as a reliable alternative.

## 211 PIWIL proteins are potential biomarkers and promising therapeutic targets in invasive breast carcinomas

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**Background:** The recently discovered (2006) Piwi-interacting RNAs (piRNAs) represent small noncoding regulatory RNAs that interact with the human four PIWIL1-4 RNA-binding proteins belonging to the Argonaute (AGO) family. PIWIL proteins are pivotal in piRNAs biogenesis and PIWI-piRNA complexes constitute genetic, epigenetic and post-transcriptional regulators implicated in genomic integrity through suppression of transposable elements, maintenance germinal and somatic differentiated and stem cells, and homeostasis *via* piRNA-mRNA induced activation or inhibition of gene expression. In a small number of recent studies, deregulated expression of PIWIL proteins and piRNAs was observed in various types of tumors and associated with several hallmarks of cancer.

**Design:** *We analyzed PIWIL1-4 expression at RNA level by quantitative RT-PCR in a large series of 526 patients who had undergone partial or complete mastectomy for unilateral invasive primary breast cancer. At protein level, study of PIWIL1-4 expression was performed by immunohistochemistry (IHC) in a series of 200 patients belonging to the large series.*

**Results:** In normal breast tissues, various profiles of PIWIL proteins were observed, with low expression of PIWIL2 and PIWIL4 at RNA and protein levels, but no expression of PIWIL1 and PIWIL3. In invasive breast carcinomas, PIWIL 1 and PIWIL 3 were upregulated (PIWIL1: Total 30%, NNN 25%, HER2+ 30%, RH+HER2- 31%, RH+HER2+ 21%; PIWIL3: Total 6%, NNN 4,2%, HER2+ 15%, RH+HER2- 4%, RH+HER2+ 25%), whereas PIWIL2 and PIWIL4 were downregulated (PIWIL2: Total 48,3%, NNN 52,5%, HER2+ 52,1%, RH+HER2- 50,3%, RH+HER2+ 25,9%; PIWIL4: Total 43,3%, NNN 28,7%, HER2+ 39,7%, RH+HER2- 47,3%, RH+HER2+ 53,4%) at RNA level. IHC analysis revealed PIWIL1 and PIWIL3 overexpression (35% and 8%, respectively) and PIWIL2 and PIWIL4 underexpression (45% and 49%, respectively) at protein level. In tumor cells, PIWIL proteins were located mainly in the nucleus and slightly in the cytoplasm as

compared to moderate nuclear staining in normal and pre-invasive lesions. Significant positive correlations were identified between PIWIL3/PIWIL4 and large tumor size, high histological grade and subtypes, advanced stage and poorer prognosis.

**Conclusions:** Deregulated PIWIL proteins with PIWIL1/PIWIL3 overexpression and PIWIL2/PIWIL4 under-expression, could contribute to breast carcinogenesis through altered levels of piRNAs and may constitute potential biomarkers and therapeutic targets.

## 212 PD-L1 Expression by Invasive Micropapillary Carcinomas of the Breast is Independent of the Degree of Tumor Infiltrating Lymphocytes

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**Background:** The breast tumor immune microenvironment (TIME) has a crucial role in tumor progression and patient outcome. Brisk stromal tumor infiltrating lymphocytes (TIL) is associated with improved outcome including overall survival in patients with invasive ductal carcinomas (IDC) and correlates with adaptive expression of the immune checkpoint ligand PD-L1 by IDC tumor cells. Invasive micropapillary carcinomas (IMPCs) are a special carcinoma subtype with poor prognosis; they are characterized by avascular tumor cell clusters with reverse nuclear polarity and retraction from the adjacent stroma. In contrast to what has been reported in IDC, increased TIL is reported to correlate with poorer outcome in patients with IMPC (Am J Clin Pathol. 2006;126:740-6); however, PD-L1 expression patterns has not been evaluated in mammary IMPC.

**Design:** Whole slide H&E sections of 12 cases of mammary carcinomas with pure (>90%) IMPC histology were assessed for the degree of stromal TIL (0=absent, <5%=focal, 5-25%= low, 25-50%=moderate, and >50%=brisk) and tumor-associated macrophages (TAM). PD-L1 immunohistochemistry was performed on whole slide sections, and membranous PD-L1 labeling by tumor cells, TIL and TAM was recorded (>1%=positive).

**Results:** All IMPC contained TAM, displayed prominently hyalinized stroma, and lacked tertiary lymphoid structures. The majority (n=9, 75%) of IMPC contained focal-low TIL, with 3 (25%) tumors containing moderate TIL. All IMPC with moderate TIL were ER-, compared to 11% of cases with focal-low TIL (p=0.03). All IMPC contained PD-L1+ TAM and PD-L1+ TIL; the majority (n=10, 83%) of IMPC had a low-moderate (10-30%) proportion of PD-L1+ TIL, with 2 (17%) tumors containing a high (75%) proportion of PD-L1+ TIL. Four (33%) IMPC displayed tumor cell PD-L1 labeling; each case contained randomly dispersed clusters of PD-L1+ tumor cells among a background of predominantly PD-L1- tumor cells. Although limited by small sample size, there was no association between the %TIL, hormone receptor status, or HER2 status and the degree of PD-L1 labeling by the TIL or tumor cells.

**Conclusions:** Mammary IMPC displays features of an immunosuppressive or immunologically "cold" TIME. In contrast to IDC, IMPC tumor cells express PD-L1 independent of the degree of TIL, suggesting a component of innate or constitutive PD-L1 expression. Further exploration of TIL subsets and therapeutic strategies that could convert an immunologically cold TIME into an immunologically active is warranted.

## 213 Telomere Length Alterations in Invasive Micropapillary Breast Carcinomas

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**Disclosures:** Karin Miller: None; Christine Davis: None; Jacqueline Brosnan-Cashman: None; Pedram Argani: None; Christopher Heaphy: None; Ashley Cimino-Mathews: None

**Background:** Mammary invasive micropapillary carcinomas (IMPC) are characterized by micropapillary cell clusters that lack fibrovascular cores and display reverse nuclear polarization with retraction from the adjacent breast stroma. IMPC has an unfavorable prognosis relative to invasive ductal carcinoma (IDC), as IMPC is more likely to present with lymph node metastases. Telomeres are nucleoprotein complexes located at the ends of chromosomes that protect against chromosomal degradation and recombination. Short dysfunctional telomeres lead to chromosomal instability, promote tumorigenesis, and are associated with poor prognosis. In other organs such as bladder, IMPC frequently contain TERT promoter mutations that lead to short telomeres; however, telomere length distribution has not been evaluated in breast IMPC.

**Design:** Histologic review of archived breast carcinomas described as having micropapillary features resulted in the identification of 12 cases with pure (>90%) IMPC histology. Whole slides were subjected to telomere-specific fluorescence *in situ* hybridization that provides

single cell resolution of telomere lengths while maintaining the tissue architecture. Relative telomere lengths were assessed by comparing the telomere signal intensities in the cancer cells to the adjacent benign cells.

**Results:** The IMPC consisted of 6 (50%) ER+/HER2-, 2 (17%) ER+/HER2+, 2 (17%) ER-/HER2+, and 2 (17%) triple negative carcinomas (TNBC). The majority (n=10, 83%) of IMPC displayed abnormal telomere lengths (9 short and 1 heterogeneous); 2 (17%) displayed normal telomere lengths. None of the cases demonstrated activation of the alternative lengthening of telomeres (ALT) pathway. The sample size precluded formal statistical comparison; however, IMPC with abnormal telomere lengths occurred in older patients (mean, 62 yrs vs 49 yrs), were larger (mean, 2.6 cm vs 1.8 cm), had higher Ki67 proliferation indices (mean, 32% vs 24%), and presented at higher stage (Stage 3; 33% vs 0%) than IMPC with normal telomere lengths. All ER-/HER2+ and TNBC cases had shortened telomeres.

**Conclusions:** Mammary IMPC telomere length alterations parallel those seen in IDC, of which >85% display abnormally short telomeres, and are in agreement with the association of short telomere lengths with HER2+ and TNBC phenotypes. Telomere length alterations may have prognostic utility in IMPC, thereby warranting further evaluation of telomere lengths in breast carcinomas with varying extents of micropapillary morphology.

## 214 A Quantitative Centrosomal Amplification Score (CAS) Predicts Local Recurrence in Ductal Carcinoma In Situ

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**Disclosures:** Karuna Mittal: None; Michael Toss: None; Guanhao Wei: None; Jaspreet Kaur: None; David Choi: None; Brian Melton: None; Remus Osan: None; Emiel Janssen: None; Havard Soiland: None; Michelle Reid: None; Padmashree Rida: None; Emad Rakha: None; Ritu Aneja: None

**Background:** Routine clinicopathologic characteristics have limited ability to predict risk of local recurrence (LR) in women with ductal carcinoma in situ (DCIS) of the breast. Furthermore, prognostic models such as Oncotype DCIS Score and Van Nuys Prognostic Index (VNPI) lack reliability and reproducibility. Thus, under- or over-treatment of DCIS is a persistent clinical problem. Here in this study, we show that the extent of centrosome amplification, a phenotype widely prevalent in breast tumors, can predict the risk of LR after lumpectomy in DCIS patients.

**Design:** We have pioneered a semi-automated pipeline that integrates immunofluorescence confocal microscopy with digital image analysis and yields a quantitative Centrosomal Amplification Score (CAS) for each patients' tumor sample by evaluating severity and frequency of centrosomal aberrations therein. To this end, discovery cohort (DC) n=133 and a validation cohort (VC) n=119 of formalin fixed paraffin embedded DCIS samples were firstly immunofluorescently stained for centrosomes followed by imaging and image analysis. Finally, generated a composite CAS score for each patient sample by integrating the numerical (CASi) and structural (CASm) aberrations.

**Results:** We observed that DCIS cases with LR exhibit significantly higher CAS than recurrence-free cases, and higher CAS is significantly associated with greater risk of developing LR (HR=7.1 for DC and 4.3 for VC, $p<0.0001$). For the high and low CAS groups for DC, the 5-year risks of recurrence were 87.1% and 12.9% respectively ($p<0.001$). CAS remained a significant independent predictor of relapse-free survival (HR=8.7 for DC and 3.4 for VC, $p<0.0001$) after accounting for potentially confounding factors like age, tumor size, comedo necrosis and radiotherapy. In a head-to-head comparison of the ability of VNPI and CAS to predict risk of LR illuminated that CAS was able to stratify the DCIS patient group into subgroups with high and low risk of LR with much higher significance ($p<0.0001$) than VNPI (HRs for CAS=7.8, vs. HR for VNPI=0.959). Among patients treated with lumpectomy alone in both the DC and VC, CAS was able to identify a subgroup of patients who are likely to benefit from adjuvant radiotherapy.

**Conclusions:** Thus, our data compellingly show that CAS quantifies the risk of LR in DCIS patients with the highest concordance, and provides a novel, innovative tool that enables treatment of DCIS patients to be tailored to their individual risk profiles.

## 215 Differential Expression of CD276, JAK2 and FOXO1 mRNA in Breast Carcinoma, Breast Cancer Cell Lines and Mammospheres

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**Background:** CD276 (B7-H3) is a membrane glycoprotein involved in immune evasion by T-cells inhibition. Recent studies have shown its role in cancer progression through regulation of cell migration, invasion and epithelial-to-mesenchymal transition via JAK2/STAT3. Moreover, its control over FOXO1 for the maintenance of cancer stem cells has also been suggested. Nevertheless, very few data in breast carcinoma (BC), in vitro studies and stem cell conditions is currently available.

**Design:** We included a non-consecutive series of 103 patients with BC: Luminal A (26%), Luminal B/HER2- (14%), Luminal B/HER2+ (25%), HER2-enriched (9%) and Triple-Negative/Basal-Like (TN/BL) (26%). We also included 1 normal mammary epithelium (184A1) and 6 BC cell lines (MCF-7, T47D, BT474, SKBR3, MDA-MB468 and MDA-MB231) representative of intrinsic phenotypes. All cell lines grew in monolayer and derived-mammospheres were obtained except for MDA-MB468. We analyzed *CD276, JAK2 and FOXO1* mRNA expression by qRT-PCR using TaqMan® Assays. We used PUM1 and β-actin as reference genes, and normal breast tissue and 184A1 cell line as control samples. Relative changes in gene expression were calculated as the fold-change by the 2–ΔΔCt method. All experiments were done by duplicates. *In vivo* results were correlated with clinic-pathological factors, and *in vitro* results were analyzed comparing the expression average and standard deviation among all BC cell lines and plotted with GraphPad Prism.

**Results:** *CD276, JAK2 and FOXO1* mRNA expression in tumor samples was seen in 75%, 75% and 24%, respectively. A positive correlation was found between all three genes (all p<0.000).Their expression was higher in Luminal A and B/HER2-negative tumors (all p≤0.003). *JAK2* correlated with histological grade 2, absence of necrosis (p≤0.028), and smaller tumor size as a trend (p=0,17). Similar results were found for *FOXO1* but only as a trend (all p>0.05). *In vitro* studies showed increased *CD276* in MCF-7 and BT474 cell lines and mammospheres; and for *FOXO1* in T47D, MCF7 and MDA-MB468 cell lines and in MCF7, SKBR3 and MDA-MB231 derived mammospheres (all p≤0,029). In contrast to tumor samples, *JAK2* was either normal or low in both cell conditions.

**Conclusions:** Our results suggest the involvement of *CD276, JAK2 and FOXO1* in the pathogenesis of Luminal BC. Further, *FOXO1* expression in SKBR3 and MDA-MB231 derived-mammospheres support its role in the maintenance of cancer stem cell phenotype.

## 216 Next-Generation Sequencing for HER2 Status in Breast Cancer: Comparison with Immunohistochemistry and in situ Hybridization by 2018 Focused Update

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**Background:** Human epidermal growth factor receptor 2 (HER2) status is routinely tested on newly diagnosed or metastatic breast cancer, to assess potential benefit from anti-HER2 therapies. In the era of precision medicine, increasing numbers of advanced stage breast cancers have been tested using next-generation sequencing (NGS) panels, which could also estimate HER2 status. Current guidelines (CAP/ASCO 2013 guideline with the 2018 focused update) address the interpretations of HER2 testing using immunohistochemistry (IHC) and in situ hybridization (ISH). However, the correlation among NGS panel, IHC, and ISH testing results is largely unknown.

**Design:** Utilizing a clinically validated 130-gene NGS panel, the HER2 status was determined in 54 breast cancer specimens (20 primary and 34 metastatic cases). Two different thresholds were set to call HER2 amplification. Clinicopathologic characteristics including HER2 IHC/ISH results were collected.

**Results:** Using the 2013 guideline, 9 cases were positive for HER2, 6 cases equivocal, and 39 cases negative. Applying the 2018 updated criteria, 2 of the positive cases reclassified as negative, one with polysomy features and the other monosomy. All 6 equivocal cases reclassified as negative, all with polysomy features. Using the 2018 update criteria and the high threshold for NGS, the sensitivity was 57.1%, specificity 100%, positive predictive value (PPV) 100%, and negative predictive value (NPV) 94%. All reclassified cases were in the negative range. When using the low NGS threshold, the sensitivity was 74.1%, specificity 95.7%, PPV 71.4%, and NPV 95.7%. The 5

reclassified cases fell into the negative range. Using NGS panel, at least one pathogenic/likely pathogenic variant was detected in 94% of cases (51 cases).

**Conclusions:** The investigated NGS panel is moderately sensitive but highly specific for detecting HER2 status. This panel provides high PPV and NPV compared to existing IHC/ISH assays. NGS testing also has the strength of providing multiple molecular data using a single test, and is particularly valuable in the setting of a limited sample.

## 217 Non-atypical and Atypical Intraductal Papilloma of the Breast Diagnosed on Core-Needle Biopsy: Upgrade Rates at Surgical Excision

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**Disclosures:** Sarah Morgan: None; Gulisa Turashvili: None

**Background:** The reported upgrade rates of non-atypical intraductal papillomas (N-IDP) diagnosed on core-needle biopsy (CNB) are 0-33% and <5% in recent studies, in contrast to up to 72% for atypical IDPs (A-IDP). While CNB diagnosis of A-IDP mandates excision, the management of N-IPD remains controversial. Follow-up has recently been suggested for screen-detected N-IPDs with concordant radiology. We aimed to determine the upgrade rates and predictors of upgrade for N-IPDs and A-IPDs diagnosed on CNB at our institution.

**Design:** We retrospectively identified CNBs with histologic diagnosis of N-IPD and A-IPD and subsequent excision specimens in 2000-2018. Clinicopathologic variables were recorded. Upgrade was defined as the diagnosis of ductal carcinoma in situ (DCIS) or invasive carcinoma in excisions subsequent to CNB diagnosis.

**Results:** There were 125 CNBs with N-IPDs from 123 patients, and 41 CNBs with A-IDPs from 40 patients. Patients with A-IDPs were older (p=0.02; median age 58 years [38-86] vs 53 years [18-83]) and had larger lesions (p=0.004; median size 2 cm [0-9] vs 1 cm [0.1-4.7]). Clinical presentation differed between N-IPDs and A-IPDs (p=0.0001): mass in 43% (54/125) vs 49% (20/41); nipple discharge in 16% (20/125) vs 10% (4/41); calcifications in 2% (3/125) vs 22% (9/41); cystic lesions in 4% (5/125) vs 5% (2/41); non-mass enhancement in 2% (2/125) vs 5% (2/41); and other lesions in 33% (41/125) vs 10% (4/41). The upgrade rate for N-IDPs was 11% (14/125), including 10 cases upgraded to DCIS and 4 cases upgraded to invasive carcinoma (2 invasive ductal carcinomas (IDC) no special type, 1 invasive lobular carcinoma and 1 mucinous carcinoma). In addition, 7% (9/125) of cases with CNB diagnosis of N-IDP showed atypical ductal hyperplasia associated with IDP in subsequent excisions. Of 41 cases with CNB diagnosis of A-IDP, 21 (51%) were classified as DCIS (n=16) or IDC (n=5) in excisions. The only predictor of upgrade was the size of lesion (N-IDPs: p=0.007, median size 1 cm [0-5] vs 2 cm [1-3]; A-IDPs: p=0.04, median size 1 cm [0-4] vs 2 cm [1-9]).

**Conclusions:** The upgrade rate for N-IDPs diagnosed on CNB was 11% which is higher than that reported in recent studies, with DCIS being the predominant diagnosis. A-IDPs had higher upgrade rates at 51%. The size of lesion was the only predictor of upgrade for both A-IDPs and N-IDPs. The small size of the radiologic target in combination with radiologic-pathologic concordance could be used to spare carefully selected patients from surgery.

## 218 Breast Cancer in Tanzanian Patients: An Assessment of Tumor Type, Grade, Biomarker status, and Tumor Infiltrating Lymphocytes

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**Disclosures:** Alex Mremi: None; Gloria Broadwater: None; Terry Hyslop: None; Allison Hall: None

**Background:** Breast cancer is one of the most common cancers in women worldwide and is a major cause of morbidity and mortality in Sub-Saharan Africa. There is evidence that the pathologic characteristics of breast cancers in African women may differ from their counterparts of European descent.

In recent years, assessment of tumor infiltrating lymphocytes (TILs) in breast cancer has gained prominence as a prognostic and predictive biomarker. We sought to characterize the pathologic features of breast cancer in a Tanzanian population, including TILs and determine whether TILs are associated with other pathologic and clinical features in this context.

**Design:** We examined consecutive cases of breast cancer in Tanzanian (TA, n=85), African American (AA, n=112) and Caucasian American (CA, n=110) women. Each case was assessed tumor type, grade and mitotic count, and TIL involvement. For TA cases, ER and HER2 immunohistochemical stains were performed in the same laboratory using the same methods that were used for the American cases as part of standard care. Demographic information and gross tumor features were extracted from the subjects' medical records. Chi-square tests were used to compare categorical factors, and Kruskal-Wallis tests were used to compare continuous factors.

**Results:** The tumors from all three groups were predominantly ductal carcinoma of no special type. TA and AA cases were more likely than CA cases to be high grade (p=0.014), ER-negative (p<0.001), and to have a high mitotic rate (p<0.0001, see table).

Higher levels of TIL involvement were seen among TA and AA subjects compared to CA subjects (TA- 20%, AA- 20%, CA-15%, p=0.008). However, when subset by ER and HER2 status, the TIL involvement in each group is similar. Among TA subjects, TIL levels are higher in ER-negative tumors.

| | Grade 1/2 | Grade 3 | Median Mitotic Rate | ER+ | HER2+ | Median TIL Involvement |
|---|---|---|---|---|---|---|
| TA | 51% | 49% | 14/10 hpf | 51% | 27% | 20% |
| AA | 53% | 47% | 9/10 hpf | 75% | 18% | 20% |
| CA | 69% | 31% | 4/10 hpf | 85% | 12% | 15% |

**Conclusions:** TA and AA subjects were more likely to have tumors with aggressive features than CA subjects. Breast cancers in TA and AA subjects also had a higher rate of TIL involvement than breast cancers in CA subjects, likely related to the increased frequency of ER-negative tumors in the Tanzanian and African American populations.

These findings suggest that treatments associated with improved outcomes in breast cancers with high levels of TILs, such as neoadjuvant chemotherapy, should be evaluated in women in Sub-Saharan Africa and in African-American women. In addition, further investigation into the molecular basis for high mitotic rates may yield insight into tumor biology in these populations.

## 219 Bilateral Breast Cancer, Synchronous and Metachronous: Clinicopathologic Characteristics and Prognostic Outcomes

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**Disclosures:** Smitha Mruthyunjayappa: None; Kui Zhang: None; Shi Wei: None

**Background:** The incidence of bilateral breast cancer (BBC) is reported to range from 1.4 to 11.8%. Women with a first primary are at a 2-6-fold increased risk of developing contralateral BC. However, there have been limited studies analyzing the clinicopathologic features of BCC and conflicting data exist on the prognostic significance of BBC.

**Design:** The clinicopathologic parameters of the primary BCs diagnosed at the authors' institution between 1998 and 2013 were recorded. Patients with stage IV BC at initial diagnosis were excluded. BCs were categorized as unilateral (UBC) or BBC while the latter were further divided into synchronous (SBBC) or metachronous (MBBC) based on the interval between the first and the contralateral BC (≤3 and >3 months, respectively). Analyses of distant relapse-free survival (RFS) and disease specific survival (DSS) were performed using the Kaplan-Meier method and the log-rank test.

**Results:** Of the 5941 patients included in the study, 110 (1.9%) were diagnosed with SBBC (n=58) or MBBC (n=52). The median time to the second tumor was 67.9 months (range, 3.1-216.5) among patients with MBBC. When compared to UBC, BBC was associated with a significantly lower rate of the ductal (no special) type, high grade, HER2-positive or node-positive disease, while no difference was found for age, race, ER/PR status, or pathologic tumor stage. Interestingly, SBBC were strongly associated with the lobular phenotype, non-high grade, and ER/PR-positive disease when compared to MBBC, whereas age, race, HER2, pathologic tumor stage and nodal status were similar between the two groups. Moreover, SBBC were associated with a significantly higher concordant rate for ER, PR, and HER2 status when compared to MBBC while no difference was found for histologic type and grade. Patients with BBC had a significantly worse RFS but a similar DSS when compared to those with UBC. Being African American, having higher pathologic tumor or node stages were significantly associated with worse RFS in patients with BBC on multivariate analysis, while nodal status was the only significant prognosticator for DSS. SBBC and MBBC had similar outcomes in all survival analyses.

**Conclusions:** Significant differences in some pathologic characteristics between BBC and UBC were identified in our cohort. BBC was associated with a shorter RFS, but a similar DSS when compared to UBC. Despite the significant difference in the receptor profiles, the prognoses of SBBC and MBBC were similar, in contrast to some earlier studies.

## 220 Characterization of LAG-3 and TIM-3 Expression in Tumor-Infiltrating Lymphocytes and on Tumor Cells as a Component of the Breast Cancer Immune Micro-environment

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**Disclosures:** Fedaa Najdawi: None; Karen Dresser: None; Sonal Jangalwe: None; Johanna Kaufmann: *Employee*, TESARO Inc.; *Employee*, TESARO Inc; Michael Brehm: None; Benjamin Chen: *Grant or Research Support*, TESARO Inc.

**Background:** The immune environment of breast carcinoma is incompletely understood. Signaling through checkpoint receptors, such PD-1, LAG3, and TIM3, may be a mechanism of immune escape for tumors, and thus are emerging targets for cancer immunotherapy. We examined these markers on tumor cells and tumor-infiltrating lymphocytes (TILs), and correlated their expression with clinicopathologic features.

**Design:** Patients were prospectively consented under an IRB-approved protocol for fresh and archival tissue, and peripheral blood. FFPE sections of invasive breast carcinoma (n=69) and DCIS (n=3) were examined by IHC using antibodies to CD3, PD-L1, PD-1, TIM3, and LAG3. CD3+ TIL density was quantified per the International TILs Working Group. Expression of each marker on tumor cells and TILs was scored, and correlated with pathologic features including tumor differentiation, ER/PR status and Her2 amplification.

**Results:** We identified a subset of breast carcinoma with high (>=30%) LAG3 (n=8, 12%) and TIM3 (n=5, 7%) on tumor cells. All 8 cases with high LAG3 on tumor cells showed low (<10%) LAG3 expression on TILs. TIL density was also low. No PD-L1 or PD-1 expression on tumor cells was appreciated, and PD-L1 expression on TILs was <1% in 7/8 cases. TIM3 was co-expressed on tumor cells in 1 case. Three cases were triple negative for ER/PR/Her2, and 4 cases were poorly differentiated. One of 3 DCIS cases (grade 3, ER+/PR+) showed high LAG3.

Tumors with high TIM3 on tumor cells showed a higher TIL density, and concurrent expression of markers on TILs including TIM3 (4 cases >=30%), LAG3 (3 cases 10-29%), PD-L1 (3 cases 1-50%,1 case >50%), and PD-1 (3 cases 10-30%). All 5 cases were poorly differentiated and 3 were triple negative. One case showed concurrent high PD-L1 on tumor cells and high TIM3 on TILs and was a metaplastic, triple negative carcinoma.

**Conclusions:** Interestingly, a subset of poorly differentiated, triple negative breast carcinomas with low TILs show LAG3+ tumor cells which could represent an actionable target for emerging checkpoint inhibitors. Cases with TIM3 tumor expression were co-infiltrated with LAG3+, TIM3+, PD-1+, PD-L1+ TILs, supporting a potential role for combination immunotherapy. This IHC analysis will be supported by flow cytometry analysis of immune cells from fresh tumor tissue and peripheral blood to gain a deeper understanding of the breast immune microenvironment. The functional and clinical consequence of LAG3 and TIM3 expression on tumor cells deserves further study.

## 221 Performance and Utility of Invasive Breast Cancer Oncotype Dx Testing on Core Needle Biopsies

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**Disclosures:** Gahie Nam: None; Kamaljeet Singh: None; Mary Lopresti: None; Madhu Ouseph: None; Li Juan Wang: None; Yihong Wang: None

**Background:** Oncotype Dx (ODx) estimates the risk of distant recurrence and helps predict the adjuvant chemotherapy benefit in estrogen receptor (ER) positive breast cancer. Most ODx data is derived from excisional specimens with limited ODx data on core needle biopsies (CNB). Our aim is to assess the performance and utility of ODx testing on CNB in neoadjuvant setting.

**Design:** Consecutive ODx results on CNB from 2012-2018 were reviewed. Clinical information and pathological parameters were recorded. Risk groups were identified based on TAILORx trial criteria. Relationship of ODx with clinico-pathological features and neoadjuvant treatment (NAT) options was analyzed.

**Results:** Total 76 cases were identified. ODx results were reported in 75/76 cases with one insufficient case. Mean patient age was 59 (range 33-84). All tumors were ER positive and Her2 negative: 54 were invasive ductal, 16 lobular and 6 with ductal and lobular features. There were 14 grade I, 50 grade II and 12 grade III tumors. ODx score was low in 36, intermediate in 29 and high in 11. There were three main purposes for performing ODx on CNB. Group 1 included 6 pts (1 T1a, 4 T1b and 1 T1c) in which ODx was performed due to limited material on the excision. Group 2 included 33 patients with mostly T1cN1 and T2 tumors and histological grade <=2 (10 IA, 18 IB, 3 IIA, 2 IIIA). ODx was performed to determine first steps in management either primary surgery or neoadjuvant systemic treatment. 15 cases with a ODx score >18 received neoadjuvant endocrine therapy while primary surgery was performed in 8 with low score. Group 3 included 36 patients (6 IA, 18 IB, 2 IIA, 2 IIB, and 2 IIIA) in which the neoadjuvant treatment was clinically chosen, ODx was performed to help decided

neoadjuvant chemo- vs endocrine therapy. Neoadjuvant endocrine therapy was selected with low score (<18), and neoadjuvant chemotherapy was selected based on score >25. Additional clinical information were used for decision marking for cases with score 18-25. 8 patients in group 2 and 5 patients in group 3 were opted out due to additional information such as genetic mutation and other factors (older age with comorbidities).

**Conclusions:** CNB material is sufficient for ODx testing. ODx performed on CNB could provide helpful tumor biology information in combination with pathologic features and clinical information in guiding treatment decisions in neoadjuvant setting.

## 222 Metaplastic Breast Carcinoma: Comprehensive Genomic Profiling of 244 Cases and Identification of Potential Therapeutic Strategies

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**Disclosures:** Nhu Ngo: *Employee*, Foundation Medicine, Inc.; Laurie Gay: *Employee*, Foundation Medicine, Inc.; Jeffrey Ross: *Employee*, Foundation Medicine, Inc.

**Background:** Metaplastic breast carcinomas (MPBC) are a diverse group of tumors which display varying degrees of epithelial and/or mesenchymal differentiation. MPBC are a subset of triple negative breast cancers (TNBC) with worse prognosis than typical TNBC. We performed comprehensive genomic profiling (CGP) to compare genomic alterations (GA) in MPBC and invasive ductal carcinoma (IDC). We aimed to identify genomic hallmarks of MPBC which may enable novel therapeutic options.

**Design:** Formalin-fixed paraffin embedded tissue from 244 MPBC and 6,540 IDC underwent hybrid-capture based next-generation sequencing of 315 genes to identify sequence and copy number alterations and rearrangements. Tumor mutational burden (TMB) was determined on 1.1 Mb of sequenced DNA and microsatellite instability (MSI) was determined using 114 loci.

**Results:** *TP53* and *PIK3CA* mutations and *MYC* amplification occurred at similar frequencies in MPBC and IDC. The remaining GA showed little overlap. Recurrent hotspot *TERT* promoter activating mutations were detected in 36% of MPBC, but in only 0.4% of IDC. MPBC showed striking loss/inactivation of tumor suppressor genes (*CDKN2A, CDKN2B, PTEN, RB1, PIK3R1, NF1*), while IDC showed amplification of oncogenes (*CCND1, ERBB2, FGF3, FGF4, FGF19, FGFR1, ZNF703*). Intermediate/high TMB was seen in 14% of MPBC and 21% of IDC. The majority of MPBC and IDC lacked microsatellite instability (MSI). (Table)

| **Tumor Type** | **Invasive ductal carcinoma** | **Metaplastic carcinoma** | |
|---|---|---|---|
| Number of Cases | 6540 | 244 | |
| Median age (range) | 53 (21-89) | 61 (21-89) | |
| **TMB (muts/1Mb)** | 79% | | **Potential Targeted Therapies** |
| Low (≤5) | 19% | 86% | ---- |
| Intermediate (6-19) | 2% | 11.5% | Immune checkpoint inhibitors |
| High (≥20) | | 2.5% | Immune checkpoint inhibitors |
| MSI-High | 0% | 0% | ---- |
| Most Commonly Altered Genes | ***TP53*** **65%** | ***TP53*** **64%** | **----** |
| | ***PIK3CA*** **51%** | ***PIK3CA*** **40%** | PI3K-AKT-mTOR inhibitors |
| | ***MYC*** **26%** | *TERT* 36% | Telomerase/TERT inhibitors |
| | *CCND1* 17% | *CDKN2A* 36% | CDK4/6 inhibitors |
| | *ERBB2* 15% | *CDKN2B* 30% | CDK4/6 inhibitors |
| | *FGF3* 15% | *PTEN* 27% | PI3K-AKT-mTOR inhibitors |
| | *FGF4* 15% | ***MYC*** **22%** | **----** |
| | *FGF19* 15% | *RB1* 16% | ---- |
| | *ZNF703* 15% | *PIK3R1* 14% | PI3K-AKT-mTOR inhibitors |
| | *FGFR1* 14% | *NF1* 11% | ---- |
| | *BRCA1* 5% | *BRCA1* 6% | PARP inhibitors |

**Conclusions:** Chemotherapy is standard treatment for TNBC, but MPBC typically respond poorly. Intermediate/high TMB, seen in 14% of our cases, make immune checkpoint inhibitors a viable option. *TERT* (telomerase) activation plays a key role in tumorigenesis in many malignancies (PMID: 2691407). 36% of our MPBC carried *TERT* promotor activating mutations. Various strategies for *TERT* inhibition are under study, including small molecules that directly inhibit enzyme activity, G-quadruplex stabilizers, telomere homolog oligonucleotides

(GROs/T-oligos) which induce DNA damage responses, and immunotherapies using dendritic cells or peptides. Frequent *CDKN2A/2B* loss in MPBC points to a role for CDK4/6 inhibitors. Of note, 6% of our MPBC had pathogenic BRCA1 mutations, an indication for PARP inhibitors. PIK3CA mutations, common in breast cancers potentiate use of PI3K-AKT-mTOR pathway inhibitors. Our dataset of 244 MPBC is the largest to date. Further analysis of the genomics will be undertaken to refine the spectrum of "MPBC" and ultimately to identify the most effective therapy for individual patients.

## 223 Desmoid-type fibromatosis of the Breast have a lower rate of CTNNB1 mutations and higher rate of APC mutations: a series of 134 tumors

Emma Norkowski[1], Julien Masliah-Planchon[1], Celine Charron Barra[2], Sophie Le Guellec[3], Martine Trassard[4], Sylvie Bonvalot[1], Philippe Terrier[5], Jean-Michel Coindre[6], Marick Lae[7]
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**Disclosures:** Sophie Le Guellec: None; Martine Trassard: None; Marick Lae: None

**Background:** Desmoid-type fibromatosis of the Breast is rare with an incidence of 0.2% of breast tumors. This fibroblastic/myofibroblatic tumor is locally aggressive, non metastazing with a variable and unpredicable clinical course. They are characterized by abnormalities of the Wnt/ beta-*catenin* pathway.

**Design:** 134 Desmoid-type fibromatosis of the breast were collected in 4 cancer centers. All cases were reviewed by a pathologist from the French Sarcoma Group (RRePS). Clinical and radiological datas were collected. All cases had *CTNNB1* exon 3 sequencing analysis on FFPE samples.

**Results:** Nuclear beta catenin expression was seen in 79% of the tumors. Expression of epithelial markers was always negative (to exclude metaplastic carcinoma). Other spindle cell tumors (phyllodes tumors, myofibroblastoma, mesenchymal tumors, PASH, scar..) were excluded. 134 tumors were studied by Sanger sequencing. 6 cases (4.5%) were not analyzable. Among 128 tumors, 84 (65.6%) had *CTNNB1*exon 3 mutations and 44 were wild-type (34.4%). NGS was performed on 28 of the wild-type tumors : *CTNNB1* mutations was detected in 3 tumors and *APC* mutations in 14 tumors.

**Conclusions:** Compared to desmoid from all locations (Le Guellec et al, 2012, Crago et al, 2015), desmoid-type fibromatosis of the breast present a lower rate of *CTNNB1* mutations using Sanger (65.6%) or Sanger+NGS (77.7%) and a higher rate of *APC* mutations (12.5%).

## 224 PD-L1, PD-1, and CTLA-4 Expression in Invasive Breast Cancer in Young Women

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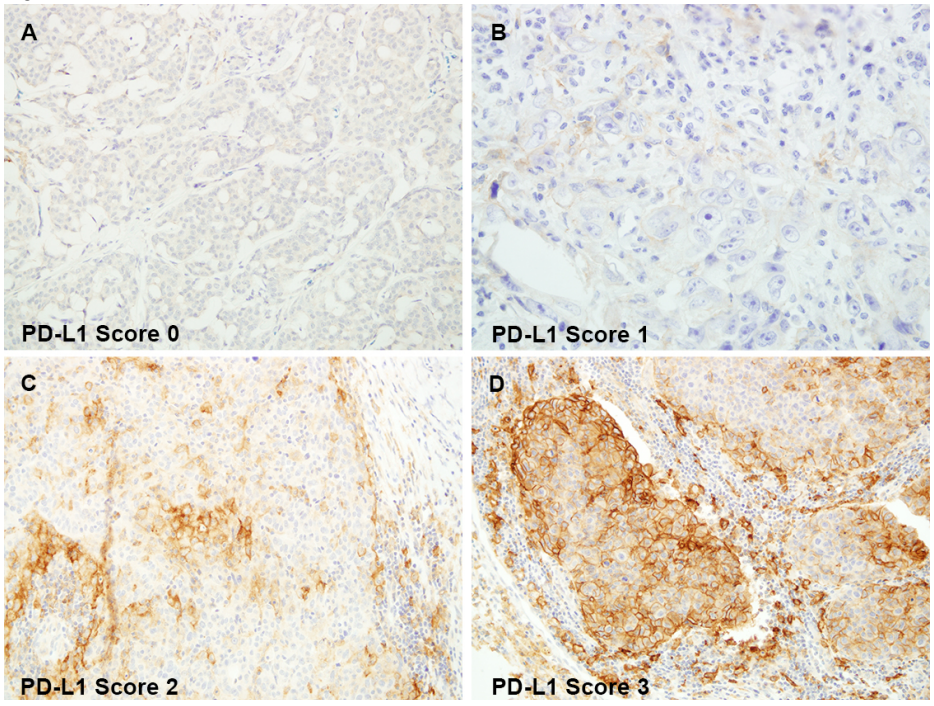
**Disclosures:** Farres Obeidin: None; Jennifer Pincus: None; K. P. Siziopikou: None; Luis Blanco: None

**Background:** Previous studies have shown that invasive breast carcinomas (IBC) in younger women are more likely to be triple negative, have higher numbers of tumor infiltrating lymphocytes, and are associated with a higher incidence of lymph node and distant metastases. Programmed cell death protein 1 (PD-1) and its ligand, PD-L1, are upregulated following activation of lymphocytes, and emerging evidence suggests that interactions between PD-1 and PD-L1 may play a key role in IBC progression and response to therapy. Tumor cells may also constitutively express PD-L1 as a result of oncogenic signaling or epithelial-mesenchymal transition. CTLA-4 is another regulatory molecule that can inhibit T-cell activation via modulation of the maturation and function of dendtritic cells. We evaluated expression of PD-1, PD-L1, and CTLA-4 in young women and compared this in older women with IBC.

**Design:** Our study population consisted of forty cases of IBC from women younger than 35 years of age and forty grade-matched IBC in women greater than 40 years. Each case was assessed for morphologic features, routine breast marker status (ER, PR, HER-2, Ki-67, and p53), number of tumor-infiltrating lymphocytes (TILs), and expression of PD-L1, PD-1, and CTLA-4 in both tumor cells and TILs. The number of TILs was graded from 1 (fewest) to 3 (greatest). PD-L1, PD-1, and CTLA-4 were assessed in both tumor cells and TILs by taking a composite score of the extent of staining multiplied by the intensity (Figure 1). A Chi-square test was used to evaluate difference in TILs while a T-test was used to evaluate PD-L1, PD-1, and CTLA-4 immunostaining.

**Results:** PD-L1 staining in both the tumor cells and TILs was significantly increased in the young women versus the controls ($p<0.005$). In addition, PD-1 was increased only in the TILs ($p<0.05$). Although the control cases were more likely to have a score of 1 for TIL number compared to a score of 3 in young women, this difference was not significant in our data set ($p=0.21$). CTLA-4 expression was similar between the two groups.

Figure 1 - 224




**Conclusions:** We found that PD-L1 expression in tumor cells and TILs and PD-1 expression in TILs was increased in our cohort of women younger than 35 years of age as compared to a grade-matched group of older women. Further sampling may demonstrate an improved relationship between absolute number of TILs and patient age, and this evaluation is in process. Our data suggests that inhibition of the PD-1/PD-L1 pathway may be a therapeutic option for IBC in young women.

## 225 Mucin Neovascularization Identified by CD31 Immunostains: A Useful Adjunct in Distinguishing Mucinous Carcinomas from Mucocele-Like Lesions in Breast Core Needle Biopsies

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**Disclosures:** Allison Onken: None; Laura Collins: None; Stuart Schnitt: None

**Background:** Neovascularization of mucin has been proposed as a potential criterion to distinguish mucocele-like lesions (MLL) from mucinous carcinomas (MC). In a prior study of 140 core needle biopsies (CNB) of mucin-producing breast lesions including MLL, mucin-producing DCIS (mDCIS) and MC, we found that mucin neovascularization identified on H&E sections was significantly more frequent in MC than in MLL and mDCIS. In the current study, we sought to determine if CD31 immunostaining to identify mucin neovascularization could be used to further refine the categorization of mucin-producing breast lesions in CNB.

**Design:** Of 140 cases from our prior study, paraffin blocks were available from 68 MLL, 16 mDCIS and 47 MC. CD31 immunostaining was performed on a representative block from each case, and immunostained slides were evaluated for mucin neovascularization, defined as the presence of CD31-positive, thin-walled microvessels within the mucin, unassociated with fibrous stroma.

**Results:** Among 131 cases with available blocks, 116 (58 MLL, 16 mDCIS and 42 MC) had remaining evaluable mucin on CD31-stained sections and form the study population. Mucin neovascularization on CD31 immunostains was significantly more frequent in MC (41 cases, 97.6%; Figure 1) than in MLL (8 cases, 13.8%; Figure 2) and mDCIS (4 cases, 25%)($p<0.00001$ for both). Among MC, the extent of neovascularization ranged from very focal to diffuse. No significant difference was noted in the frequency of mucin neovascularization between MLL and mDCIS ($p=0.28$). The sensitivity, specificity, PPV and NPV of CD31-positive microvessels within mucin for categorizing a lesion as an MC were 97.6%, 86.2%, 83.7% and 98.0%, respectively.

Figure 1 - 225

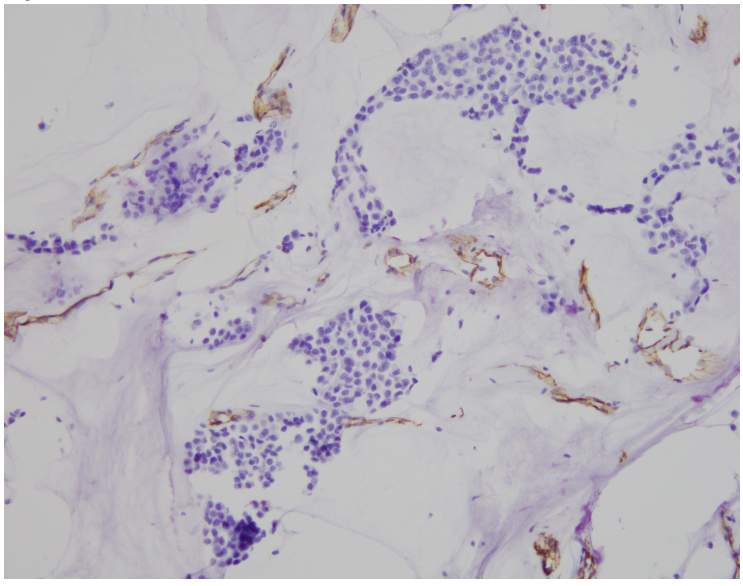
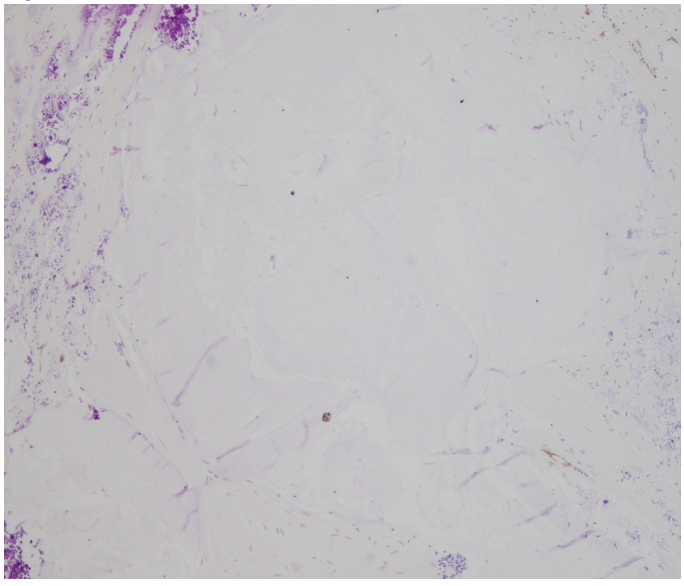


Figure 2 - 225



**Conclusions:** In our prior H&E study, mucin neovascularization had a sensitivity, specificity, PPV and NPV of 69.2%, 85.9%, 78.3% and 79.2%, respectively for categorizing a lesion as MC. In the current study, CD31 immunostaining resulted in substantial improvements in sensitivity, PPV and NPV. We conclude that CD31 immunostaining to identify mucin neovascularization is a useful adjunct for categorizing mucin-producing breast lesions in problematic CNB cases. Given the strong association of mucin neovascularization with MC, apparent MLL with CD31-positive vessels on CNB may warrant excision for definitive categorization. Conversely, a CD31 stain negative for mucin neovascularization does not entirely exclude MC, since CD31-positive vessels may be scant in some cases; clinical and radiologic correlation remain important.

## 226 Matrix Metalloproteinase 9 (MMP9) In Breast Cancer: A Marker of Poor Prognosis

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**Disclosures:** Nnamdi Orah: None; Chitra Joseph: None; Sasagu Kurozumi: None; Emad Rakha: None; Sami Alsaeed: None; Andrew Green: None; Ian Ellis: None; Islam Miligy: None; Yousif Kariri: None; Mohammed Aleskandarany: None; Ibraheem Ishankyty: None

**Background:** The extracellular matrix comprises a network of structural proteins, and its reorganization is essential during cancer progression. Matrix metalloproteinase 9 (MMP9) plays a key role in the degradation of the extracellular matrix in various cancers and is considered to play a key role in breast cancer (BC) metastasis. This study aims to evaluate the biological and clinical importance of MMP-9 in BC utilizing large BC cohorts with long-term follow up.

**Design:** *MMP9* gene copy number alteration (CNA) and mRNA expression were assessed in the METABRIC (n=1980) cohort and externally confirmed in BC Gene miner v4.0 (n=5451). Primary BC tissue microarrays (n=675) were immuno-stained for MMP9 and expression patterns correlated with clinico-pathological and molecular variables.

**Results:** *MMP9* CNA was significantly correlated with tumour size (p=0.046), histological grade (p=0.001), and Luminal B tumors (p<0.0001; *Table1*). Patients with an *MMP9* gain had a significantly worse prognosis compared to *MMP9* CNA neutral group (p=0.0076). *MMP9* mRNA expression was significantly associated with negative estrogen (ER)/progesterone receptor (PR) status (p<0.0001), high grade (p<0.0001), basal-like tumors (p<0.0001; *Table2*) and poor patient outcome (p=0.0036; *Figure1*). MMP9 protein showed significant cytoplasmic immunoreactivity. Similar to mRNA expression, high MMP9 protein expression was associated with negative ER/PR status (p<0.005), high grade (p<0.0001), high mitotic scores (p<0.0001), poor tubule formation (p=0.017), high nuclear pleomorphism (p<0.0001) and poor Nottingham Prognostic Index (p<0.0001; *Table3*). Increased MMP9 protein expression was significantly correlated with features of aggressive phenotype including high Epidermal Growth Factor Receptor (EGFR) (p=0.031) and p53 (p=0.007). Pooled *MMP9* gene expression data in BC-GenExMiner confirmed the association of high expression with poorer outcome (p<0.0001) and corroborates with the protein expression results. Cox proportional multivariate analysis showed that MMP9 protein expression (p=0.013, HR=0.68, 95% CI=0.5-0.9; *Figure2*) was an independent indicator of poor patient outcome independent of other variables.

| Factors | | Expression of MMP9 protein (cytoplasmic) | | |
|---|---|---|---|---|
| | | Low (%) | High (%) | p-value* |
| ER | Negative | 107 (59.1) | 74 (40.9) | **< 0.0001** |
| | Positive | 373 (76.0) | 118 (24.0) | |
| PR | Negative | 182 (65.7) | 95 (34.3) | **0.005** |
| | Positive | 284 (75.7) | 91 (24.3) | |
| Histological grade | 3 | 225 (64.3) | 125 (37.7) | **< 0.0001** |
| | 1,2 | 253 (79.6) | 65 (20.4) | |
| Mitotic activity | 3 | 193 (63.3) | 112 (36.7) | **< 0.0001** |
| | 1,2 | 267 (78.3) | 74 (21.7) | |
| Tubule formation | 3 | 276 (67.6) | 132 (32.4) | **0.017** |
| | 1,2 | 184 (77.3) | 54 (22.7) | |
| Nuclear pleomorphism | 3 | 258 (64.3) | 143 (35.7) | **< 0.0001** |
| | 1,2 | 201 (82.4) | 43 (17.6) | |
| Nottingham Prognostic Index | GPG | 155 (84.2) | 29 (15.8) | **< 0.0001** |
| | MPG | 253 (67.5) | 122 (32.5) | |
| | PPG | 72 (64.3) | 40 (35.7) | |

*The P values are resultant from Pearson ?2 test of association.

**Table 3 Relationship of MMP9 protein with clinicopathological characteristics**

**Conclusions:** High MMP9 expression identified a subgroup of BC with aggressive behavior and provides independent prognostic information. Thus, further studies are necessary to reveal the molecular mechanism of MMP9 to identify the potential therapeutic targets.

## 227 Utility of IBM Watson Visual Recognition Platform in Breast Pathology Images

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**Disclosures:** Ugur Ozerdem: None

**Background:** Since the launch of the Visual Recognition Tool in 2016, users in many fields have been utilizing IBM Watson Visual Recognition Platform. Enabling many of these users are Custom Classifiers, a feature that allows users to train this platform on any visual content. In this pilot investigation, Custom Classifier is first trained, and then tested on digital breast pathology images to elucidate whether basic breast microscopic image classes can be recognized by machine learning.

**Design:** IBM Watson Visual Recognition tool is trained with digital breast pathology images obtained at standardized settings in JPEG format. Four custom visual recognition classifiers are generated using a training set of images. Custom Model 1 (CM1) is generated to differentiate invasive carcinoma, in situ carcinoma (DCIS or LCIS), and benign breast tissue. Custom Model 2 (CM2) is generated to differentiate hormone receptor positive cancers from hormone receptor negative cancers in immunostain images. Custom Model 3 (CM3) is generated to differentiate HER2 positive cancers from non-positive counterparts (2+, 1+, 0) in IHC. Custom Model 4 (CM4) is generated to differentiate lymph node with macrometastasis from lymph node without metastasis. As seen in Table; 364 images are used for training CMs, and 441 images are tested using CMs. Tested images yield a confidence score (CS) between 0 and 100. Confidence scores (CS) are unitless and are neither percentages nor probabilities; i.e. they do not add up to 100, but considered comparable indicators. Confidence scores in each class in each custom model are statistically compared using Prism 7.0 software.

**Results:** The confidence scores in each custom model are significantly different between classes tested (Table). The results suggest that IBM Watson Visual Recognition tool result in different CS for invasive carcinoma, in situ carcinoma, benign breast tissue (Friedman test P<0.0001) on H&E slides, hormone receptor positive and negative cancers (Wilcoxon test P<0.0001) on IHC slides, HER2-positive and non-positive cancers on IHC slides (Kruskal-Wallis test P<0.0001), and axillary lymph nodes with macrometastasis and benign axillary lymph nodes (Wilcoxon test P<0.0001) on H&E slides.

| IBM Watson Custom Visual Recognition Model | Number of classes | Number of images used in training | Class1Tested | Class2Tested | Class3Tested | Class4Tested |
|---|---|---|---|---|---|---|
| CM1 (H&E) | 3 | 113 | Invasive carcinoma n=153 CSinv: 90.21 CSinsitu:2.56 CSbbt:1.03 P<0.0001 | In situ carcinoma n=30 CSinsitu:87.17 CSinv: 5.26 CSbbt: 9.73 P<0.0001 | Benign breast tissue n=30 CSbbt: 90.30 CSinv: 1.03 CSinsitu:2.56 P<0.0001 | N/A |
| CM2 (Hormone receptors immunohistochemistry) | 2 | 36 | Hormone receptor positive n=64 CSpos:91.13 CSneg:3.96 P<0.0001 | Hormone receptor negative n=15 CSneg:91.0 CSpos:5.6 P<0.0001 | N/A | N/A |
| CM3 (HER2 immunohistochemistry) | 4 | 41 | HER IHC 3+ Positive n=32 CSpos: 92 P<0.0001 | HER IHC -0 n=24 CSpos:1.917 | HER IHC-1+ n=29 CSpos:1.621 | HER2 IHC2+ n=19 CSpos:34.63 |
| CM4 (H&E axillary lymph node) | 2 | 174 | Positive LN n=20 CSpos:90.85 CSneg:6.45 P<0.0001 | Negative LN n=25 CSneg:91.92 CSpos:0.60 P<0.0001 | N/A | N/A |

**Conclusions:** In this pilot investigation IBM Watson Visual Recognition Platform displays statistically significant results in recognizing basic breast pathology images. These findings suggest it is worth exploring machine learning as an ancillary tool to screen and prioritize images.

## 228 Impact of recent 2018 ASCO-CAP HER2 testing guidelines on 2713 breast cancer cases treated according to 2013 ASCO-CAP HER2 guidelines- Western Indian tertiary cancer centre experience

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**Background:** The recent 2018 ASCO/CAP guidelines have provided an algorithm wherein the associated immunohistochemical finding is included in the final HER2 gene status assessment by FISH with deletion of the FISH equivocal group. We sought to see the impact of these changes on patients reported by 2013 ASCO/CAP guidelines.

**Design:** We retrospectively evaluated the consecutive cases of breast carcinoma that underwent HER2 testing by FISH by PathVysion (Abbott Molecular Inc., Des Plaines, IL, USA) and ZytoLight SPEC ERBB2/CEN 17 Probe (Zytovysion, Bremerhaven, Germany) at the Division of Molecular Pathology of our institution over a duration of 5 years between October 2013- June 2018. The cases were interpreted by expert breast pathologists according to 2013 ASCO/CAP guidelines. FISH equivocal cases were further reflex tested using the alternate, non-centromeric FISH probe by ZytoLightSPEC/D17S122. The FISH test results with HER2/CEP 17 >2, mean HER2 copy number <4 (Group A), HER2 CEP17 <2, mean HER2 copy number >6 (Group B) and HER2/CEP17 <2, mean HER2 copy number ?4-<6 (Group C, FISH equivocal) were further interpreted applying the ASCO/CAP 2018 guidelines.

**Results:** A total of 2764 breast cancer cases were evaluated by FISH in this study period. Of the 2713 (98.2%) interpretable cases, 995 (36.6%) were amplified [Group A cases (Ratio>2, Her2 <4) – 65 (6.5%) and Group B cases (Ratio <2, Her2 >6) – 9 (0.9%)], 1662 (61.3%)

were non-amplified and 56 (2.1%) (Group C) were equivocal. By immunohistochemistry, 100% of group-A and group C cases and 66% (5/9) of group -B were IHC equivocal and 44% (4/9) of group-B were IHC score 3+. According to 2018 guidelines, of the interrogated 130 cases, all group A (n=65) and group C cases (n=56) were non-amplified, while group B (n=9) retained the amplified status. With a available follow-up of 6-73 months, trastuzumab was taken by 53% (35/65) of group A (3-progressed and 4 -partial response), 88.9% (8/9) of group B (2- progressed) and 41% (14/34*- FISH reflex testing positive) of group C (2 -progressed, 1- stable). Cardiac toxicities developed in 5 cases.

**Conclusions:** The 2018 has impacted patients in group >2 and her2 <4 with over treatment in121/130 (93.1%) patients.

The group-A and group-C cases accounted for 4.5% of breast cancers, none of which were positive (Score 3+) by IHC which indirectly validates the 2018 which appears to be a welcome change.

## 229 Immunohistochemical Assessment of HRAS Q61R Mutations in Breast Adenomyoepitheliomas

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**Disclosures:** Fresia Pareja: None; Ana Paula Martins Sebastiao: None; Felipe Geyer: None; Hannah Wen: None; Achim Jungbluth: None; Zsuzsanna Varga: None; Juan Palazzo: None; Brian Rubin: None; Ian Ellis: None; Edi Brogi: None; Emad Rakha: None; Britta Weigelt: None; Jorge Reis-Filho: *Advisory Board Member*, Voliton Rx; *Advisory Board Member*, Paige.Ai; *Consultant*, Goldman Sachs

**Background:** Breast adenomyoepitheliomas (AMEs) are uncommon lesions with dual epithelial and myoepithelial differentiation. Most estrogen receptor (ER)-positive AMEs have mutations in PI3K pathway genes, whereas up to 60% of ER-negative AMEs harbor concurrent mutations affecting the *HRAS* Q61 hotspot codon and PI3K pathway genes. Here, we sought to determine the sensitivity and specificity of immunohistochemistry (IHC) with a Q61R mutant HRAS-specific antibody for the detection of *HRAS* Q61R mutations in breast AMEs, and the differences between HRAS Q61R IHC-positive and IHC-negative AMEs.

**Design:** 26 AMEs previously subjected to massively parallel sequencing (n=21) or Sanger sequencing (n=5) for *HRAS, PIK3CA*, *AKT1* and *PIK3R1* were included in this study. The ER status of all AMEs was assessed by IHC as per ASCO/CAP guidelines. Two pathologists conducted a histologic analysis and all AMEs were subjected to IHC using an HRAS Q61R-specific antibody. Any cytoplasmic and/or membranous reactivity for HRAS Q61R was considered positive. IHC was performed with observers blinded to the results of the sequencing analysis.

**Results:** 35% (9/26) of the AMEs studied harbored *HRAS* mutations, of which 78% (7/9) displayed the Q61R hotspot mutation. 19% (5/26) of AMEs expressed the HRAS Q61R protein by IHC, all of which were ER-negative and harbored the Q61R hotspot mutation by sequencing analysis. 60% (3/5) of HRAS Q61R IHC-positive AMEs displayed immunoreactivity in both, the epithelial and myoepithelial components, whereas HRAS Q61R immunoreactivity was restricted to the myoepithelium in 40% (2/5) of cases. Of the seven AMEs with *HRAS* Q61R mutations, five (71%) were positive by IHC, whereas none of the AMEs lacking *HRAS* Q61R mutations (n=17) displayed any HRAS Q61R protein expression by IHC. IHC for HRAS Q61R displayed a sensitivity and specificity for the detection of *HRAS* Q61R mutations of 71% and 100%, respectively. HRAS Q61R IHC assessment did not detect the presence of Q61K *HRAS* mutations (0/2). AMEs expressing HRAS Q61R by IHC displayed co-occurring *PIK3CA* (n=2) or *PIK3R1* (n=3) mutations, and an association with higher Ki67 index (p=0.01), i

**Conclusions:** IHC analysis of HRAS Q61R displays a high specificity and moderate sensitivity for the detection of *HRAS* Q61R mutations in breast AMEs, and it appears not to detect *HRAS* Q61K mutations. Given the rarity of *HRAS* Q61R mutations in breast lesions other than ER-negative AMEs, IHC analysis of HRAS Q61R may represent a useful marker in the diagnostic workup of these lesions.

## 230 Recurrent MED12 Exon 2 Mutations in Benign Breast Fibroepithelial Lesions in Adolescents and Young Adults

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**Disclosures:** Fresia Pareja: None; Arnaud Da Cruz Paula: None; Melissa Murray: None; David Brown: None; Edaise Da Silva: None; Dilip Giri: None; Ana Paula Martins Sebastiao: None; Britta Weigelt: None; Jorge Reis-Filho: *Advisory Board Member*, Volition Rx; *Advisory Board Member*, Paige.AI; *Consultant*, Goldman Sachs; Edi Brogi: None

**Background:** The genomic landscape fibroadenomas (FAs) and phyllodes tumors (PTs) in adults has been well characterized, whereas data on fibroepithelial lesions (FELs) in younger patients is scarce. In the adult population, FAs and PTs have been shown to harbor recurrent somatic *MED12* exon 2 mutations, and *TERT* promoter hotspot mutations have been documented in PTs. We sought to determine the frequency of *MED12* exon 2 and *TERT* promoter hotspot mutations in FAs and benign PTs in adolescents and young adults.

**Design:** Benign FELs from adolescents and young adults were centrally reviewed by three pathologists and classified according to the WHO criteria as FAs or benign PTs. The stromal component of the FELs was microdissected, and the extracted DNA was subjected to Sanger sequencing analysis of exon 2 of *MED12* and the *TERT* promoter hotspot locus. The effect of the mutations identified was assessed using a combination of functional prediction algorithms.

**Results:** Our series comprised 29 benign FELs, including 21 consecutive FAs and 8 consecutive benign PTs from adolescents and young adults, with a median age of 19 years at diagnosis (range 13-26). We identified *MED12* exon 2 mutations in 62% (13/21) of FAs and 88% (7/8) of benign PTs, and no *TERT* promoter hotspot mutations. Five FAs (24%) harbored missense mutations in the hotspot codon 44 of *MED12*, including G44D (n=3), G44S (n=1) and G44R (n=1). Seven FAs (33%) had *MED12* exon 2 in-frame deletions, out of which 5 encompassed codon 44. One FA (5%) harbored a splice site mutation in intron 1 of *MED12*. Two benign PTs (25%) harbored *MED12* exon 2 missense mutations (G44D and G44C) and five (63%) benign PTs harbored *MED12* exon 2 in-frame deletions, four of which encompassed codon 44. All identified mutations in FAs in benign PTs were predicted to be deleterious. *MED12* exon 2 mutations displayed an association with pericanalicular growth pattern in FAs ($p<0.001$, Fisher's exact test), and the only *MED12*-wild type benign PT did not differ histologically from *MED12* exon 2-mutant benign PTs.

**Conclusions:** Our study supports the notion that benign FELs in the juvenile population are underpinned by recurrent *MED12* exon 2 mutations and lack *TERT* promoter hotspot mutations, akin to benign FELs in adults. Further studies are warranted to define the genetic alterations driving benign FELs lacking *MED12* mutations in adolescents and young adults.

## 231 Immunohistochemical Analysis of IDH2 R172 Hotspot Mutations in Breast Papillary Neoplasms

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**Disclosures:** Fresia Pareja: None; Achim Jungbluth: None; Thais Basili: None; John Lozada: None; Arnaud Da Cruz Paula: None; Dilip Giri: None; Emad Rakha: None; Felipe Geyer: None; Britta Weigelt: None; Jorge Reis-Filho: *Advisory Board Member*, VolitionRx; *Advisory Board Member*, Paige.AI; *Consultant*, Goldman Sachs; Edi Brogi: None

**Background:** Solid papillary carcinoma with reverse polarity (SPCRP) is a vanishingly rare subtype of breast cancer with a distinctive morphology, reminiscent to that of the tall cell variant of papillary thyroid carcinoma. Given their rarity, differential diagnosis of SPCRPs from other breast papillary neoplasms may be challenging. SPCRPs are underpinned by recurrent *IDH2* R172 hotspot mutations or *TET2* mutations, co-occurring with mutations affecting PI3K pathway genes. Here, we sought to determine the sensitivity and specificity of IDH2 R172 immunohistochemical (IHC) analysis for the detection of *IDH2* R172 hotspot mutations in breast papillary neoplasms.

**Design:** Our series comprised 13 breast papillary neoplasms including 3 SPCRPs, 5 solid papillary/ neuroendocrine carcinomas (SPC/NECs), and 5 intraductal papillomas (IDPs). All SPCRPs and SPCs/NECs had been previously subjected to Sanger sequencing for *IDH2* R172 hotspot mutations. We conducted an IHC analysis for the detection of *IDH2* R172 mutations with a monoclonal antibody for IDH2 R172S (clone 11C8B1) in formalin-fixed paraffin-embedded tissue from 12 excision specimens and 2 core biopsies (1 SPCRP and 1 IDP). The IHC analysis was performed by two pathologists blinded to the results of the *IDH2* sequencing analysis.

**Results:** All (3/3) SPCRPs included in this study harbored *IDH2* R172 hotspot mutations, two of which harbored the R172T and one the R172S variant. All SPCRPs (3/3; 100%) were positive for IDH2 R172 by IHC, displaying a moderate to intense granular cytoplasmic immunoreactivity. All five breast SPCs/NECs lacked *IDH2* R172 hotspot mutations and did not express IDH2 R172 protein by IHC (0/5; 0%). In addition, all five IDPs included in this study lacked IDH2 R172 protein expression by IHC (0/5; 0%). In the context of breast papillary lesions, both the specificity and sensitivity of IDH2 R172 IHC analysis for the detection of *IDH2* R172 mutations are 100%.

**Conclusions:** Our results lend further support to the notion that *IDH2* R172 hotspot mutations are pathognomonic for SPCRPs in a breast specific context and show that IHC assessment of IDH2 R172 is a sensitive and specific test for the detection of *IDH2* R172 hotspot mutations in SPCRPs. Larger series of SPCRPs need to be subjected to IDH2 R172 IHC analysis to assess whether it may be employed as an ancillary test, in excision specimens or core biopsy material, to aid in the differential diagnosis of SPRCPs from other breast papillary lesions.

## 232 Exploring Mutations in the Parkinson's-Related LRRK2 RAS/GTPase Kinase in Breast Cancer, Insights from The Cancer Genome Atlas and Whole Transcriptome Sequencing

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**Disclosures:** Edgardo Parrilla Castellar: None

**Background:** Mutations in *LRRK2* are the most common genetic cause of Parkinson's disease (PD). LRRK2 is a member of the leucine-rich repeat kinases and is functionally implicated in mitochondrial homeostasis. Interestingly, women who are *LRRK2* mutation carriers are also at increased risk for breast cancer. However, there is limited information on the pathophysiologic relationship between LRRK2 and breast cancer.

**Design:** The Genomic Data Commons Data Portal (https://portal.gdc.cancer.gov) from The Cancer Genome Atlas was searched for *LRRK2* single nucleotide variants and small insertions/deletions in 3,681 unselected cases of breast cancer. Digital histopathology was performed using Slide Image Viewer. The curated list of variants was supplemented with LRRK2 variants identified from whole transcriptome sequencing performed on breast tumor tissue from a subset (n = 6) of patients from an internal trial.

**Results:** 23 *LRRK2* mutations were detected in 21 of 3,687 breast cancer cases (age at diagnosis range: 34-90 yrs.; median: 60 yrs.), including 14 missense, 4 nonsense, 4 frameshift, and 1 splice-site variant. All stop-gain mutations predicted a protein-product with truncated kinase domain. Missense variants most frequently involved the GTPase (p.S1445F and p.A1490V) or the regulatory C-terminal-of-Roc (COR) domain (p.I1691T, p.R1771T and p.G1819R)(5 of 14), whereas 3 variants localized to the kinase domain (p.S1954F, p.E2108K and p.S2058L). Although common PD variants at p.G2019 and p.R1441 were not identified, a single PD-associated mutation was present (p.G2385R). Two cases demonstrated apparent bi-allelic substitutions (p.[A306V];[S2058] and p.[ N1089S0];[ A1490V]). Nottingham grade 2-3 invasive ductal carcinoma with apocrine features was the most common histology (9 of 21 cases), albeit there were 2 additional cases of pleomorphic lobular carcinoma with granular, eosinophilic cytoplasm.

**Conclusions:** Somatic *LRRK2* mutations occur in breast cancer, with some overlap of PD-associated variants. The finding of truncating, loss-of-function and bi-allelic missense *LRRK2* mutations may indicate tumor suppressor function in this pathologic context. Frequent targeting of the GTPase/COR and kinase domains in LRRK2 identify these as mechanistically important in neoplasia and potentially targetable therapeutically. These findings, along with frequent apocrine morphology suggest that mitochondrial dysfunction may play a pathophysiologic role in this subset of breast cancers.

## 233 'Granular/Punctate' Pattern of Nuclear Immunoreactivity with Estrogen Receptor Clone 6F11 in Higher Grade Breast Carcinoma Could Represent False-Positive Staining

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**Disclosures:** Ami Patel: None; Bing He: None; Syed Hoda: None

**Background:** Per ASCO/CAP guidelines, a carcinoma is positive for estrogen receptor (ER) when ≥1% of tumor cell nuclei are immunoreactive. A peculiar 'granular/punctate' staining (GPS) with ER clone 6F11 (ER/6F11) has been regarded as 'false-positive' (Letter, Rakha et al, *J Clin Oncol* 2012;30:2929). GPS with ER/6F11 is neither widely recognized nor has it been formally studied.

**Design:** Archived cases (2011-2018) with GPS utilizing ER/6F11 (mouse IgG1) were identified. ER/SP1 (rabbit IgG) was repeated on corresponding sections. IHC staining was performed using ER/6F11 (Leica, Buffalo Grove, IL) & ER/SP1 (Ventana/Roche, Tucson, AZ) on formalin-fixed (>6 hours), paraffin-embedded sections using a modified Leica protocol. Sections were incubated in a Tris-EDTA buffer (pH = 9, epitope retrieval solution 2) at 100°C for 20 min for ER/6F11 & 10 min for ER/SP1, followed by incubation with ER/6F11 or ER/SP1 for 15 min at room temperature. ER was detected using DAB as chromogen with counterstaining with Mayer's hematoxylin using Bond Polymer Refine Detection System (Leica). Appropriate controls were used. All slides were reviewed by 2 pathologists. Pathological & biomarker data were assessed.

**Results:** 94 cases with GPS utilizing ER/6F11 were identified (**Figure 1**). 49% (46/94) were needle core biopsies and the remainder excisions. In these cases, 1-10% of tumor cell nuclei showed GPS, & were previously interpreted as weak (+). By ER/SP1, 68% (64/94) were ER:(-), i.e. showed <1% staining (**Figure 2**). ER/SP1 showed: weaker reactivity (in intensity/proportion) than ER/6F11 in 4% (4/94), similar reactivity to ER/SP1 in 23% (22/ 94), & stronger reactivity than ER/SP1 in 4% (4/94) (**Table 1**).

Invasive (inv) ductal carcinomas (ca, >1 mm) comprised the majority of GPS cases (84%, 79/94). Of these cases, 84% (66/79) were Nottingham grade 3, 68% (54/79) were PR:(-), 84% (66/79) had high (>15%) Ki67 proliferation rate, & 76% (60/79) were HER2:(-). Tumor cell nuclei were high grade in 75% (6/8) of microinvasive ca, in 75% (3/4) in other invasive ca, and in 67% (2/3) in DCIS. Oncotype DX, in one inv ductal ca (recurrence score: 66) was triple (ER, PR & HER2) negative.

| Immunoreactivity of ER/SP1 compared to GPS in ER/6F11 | | | | | |
|---|---|---|---|---|---|
| | n | Negative with ER/SP1 | Weaker with ER/SP1 | Similar to ER/SP1 | Stronger with ER/SP1 |
| **DCIS** | 3 (3%) | 2/3 (67%) | 0 | 1/3 (33%) | 0 |
| **Microinvasive ca** | 8 (9%) | 6/8 (75%) | 0 | 2/8 (25%) | 0 |
| **Inv ductal ca** | 79 (84%) | 52/79 (66%) | 4/79 (5%) | 19/79 (24%) | 4/79 (5%) |
| **Inv ca, other types** | 4 (4%) | 4/4 (100%) | 0 | 0 | 0 |
| **Total** | **94** | **64/94 (68%)** | **4/94 (4%)** | **22/94 (23%)** | **4/94 (4%)** |

Figure 1 - 233

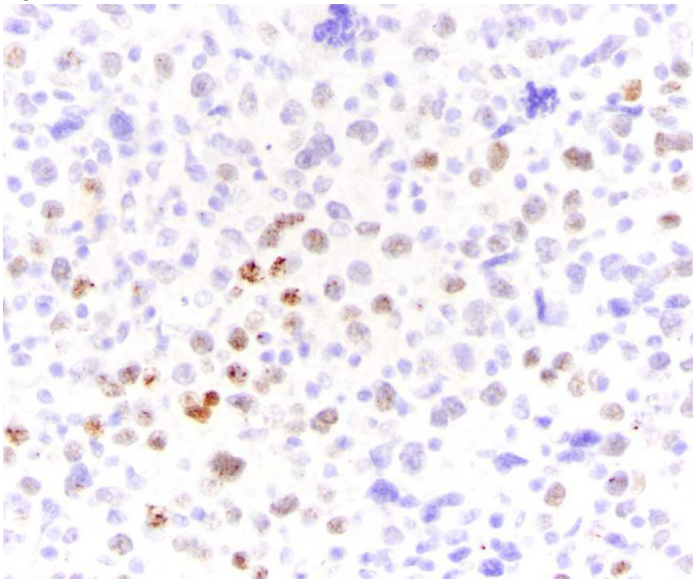
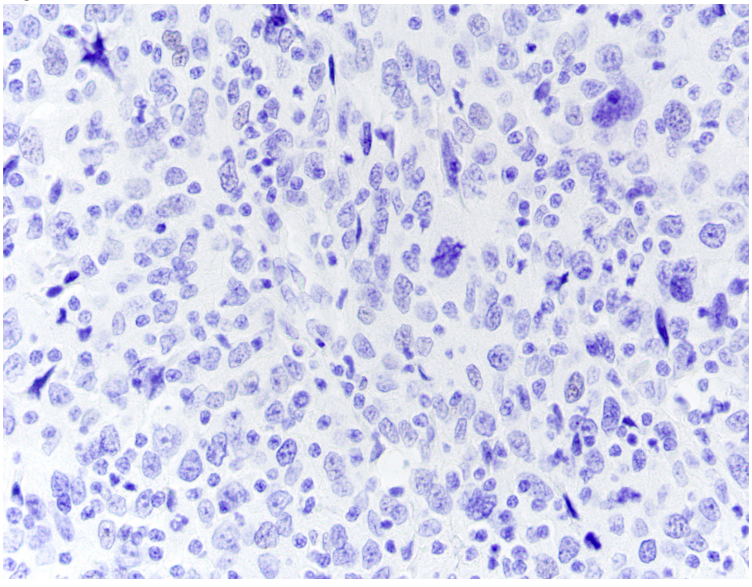


Figure 2 - 233



**Conclusions:** 68% (64/94) of cases showing GPS with ER/6F11 were not confirmed to be (+) with ER/SP1. Most 'ER discordant' cases were grade 3 inv ductal ca, PR:(-) & HER2:(-). A ER (+) result based only on GPS should be reported with caution. Further studies could explain this apparent discrepancy.

## 234 Systematic Evaluation of Glycoreceptor Binding in 2.800 Breast Cancer Cases

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**Background:** The BMBF-funded project "GlyCan" (031B0066A) aims at systematically evaluating the utility of glycoreceptor (GR) staining (Nollau et al. J Histochem Cytochem. 2013; 61(3):199-205) in breast cancer. The tumor microenvironment contains cells of the innate immune system such as macrophages and dendritic cells, which express receptors binding to defined glycan structures of the tumor cell membrane. We applied recombinant human GR to tissue sections of breast cancer specimens. The recognition of tumor cell glycans by the recombinant GR should provide information on the interaction of cancer cells with the immune system.

**Design:** We constructed 34 Tissue Microarrays (TMA) from more than 2.800 cases which were intended to represent the most common or "interesting" combinations of clinically established risk parameters, complemented by 500 consecutive and 400 cases that had not received cytotoxic chemotherapy (see **Table 1** for groups and inclusion criteria). Cases were selected out of more than 10.000 patients (all with

informed consent, follow-up and relevant clinical data). The TMA were examined for the presence of GR staining (CD301, CLEC4C, CLEC18A, DCSIGNR, Langerin, LSECtin, SRCL). Clear-cut detectable staining could be determined mainly in the cytoplasm and/or membrane, rarely in the nucleus of tumor cells depending on the respective GR usually with a neglectable level of background.

**Results:** The distribution of positivity of GR across the different TMA is depicted in **Figure 1**. CD301 showed the most prominent positive staining in almost half of the cases while triple negative breast cancer expressed the broadest spectrum of glycans. Preliminary analysis revealed that the majority of GR (with the exception of CD301 and SRCL) did not show any staining in grade 1 tumors. Estrogen receptor (ER) negative cases were detected by CLEC4C, while CLEC18A showed staining exclusively in ER positive cases. An initial survival analysis revealed that CLEC18A positive cases tend to be without recurrence or death due to cancer. In contrast DCSIGNR positive cases showed a significantly unfavorable prognosis in comparison to all other cases.

| Category | Group | Inclusion criteria | No. of spots | Total no. |
|---|---|---|---|---|
| **Consecutive cohort** | Consecutive cohort | ≤70 years, ER+, Events ≥ 10% | 500 | 500 |
| **No chemo** | TamOnly | NST, ≤70 years, G2, ≤ pT2, ER+, **no chemo**, Events ≥ 10% | 400 | 400 |
| **Gray zone** | Gray zone | NST, ≤70 years, **G2, pT1b/1c/2, N0, ER+**, Events ≥ 10% | 300 | 300 |
| **Gray zone like** | G1-Gray zone | NST, ≤70 years, **G1**, **pT1b/1c**, **N0**, **ER+**, Events ≥ 10% | 400 | 575 |
| | G3-Gray zone | NST, ≤70 years, **G3**, **pT2**, **N+, ER+**, Events ≥ 10% | 175 | |
| **Age cohorts** | old "light" | NST, **>70 years**, **G2, pT1c, N0, ER+**, Events ≥ 10% | 100 | 345 |
| | old | NST, **≥85 years**, Events ≥ 10% | 100 | |
| | young | NST, **≤35 years**, Events ≥ 10% | 92 | |
| | young vs. old | NST, **≥90 vs. ≤30 years**, Events ≥ 10% | 53 | |
| **The "unusual"** | Disproportionates I | NST, ≤70 years, **G3**, pT2, **N0**, ER+, Events ≥ 10% | 100 | 200 |
| | Disproportionates II | NST, ≤70 years, **G1**, pT1c, **N+**, ER+, Events ≥ 5% | 100 | |
| **Special Types** | Lobular (Special Type 1) | **Lobular**, ≤70 years, G2, ≤ pT2, N0, ER+, Her2-, Events ≥ 10% | 200 | 312 |
| | Tubular (Special Type 2) | **Tubular**, ≤70 years, ≤pT2, ER+, Events ≥ 10% | 60 | |
| | Mucinous (Special Type 3) | **Mucinous**, ≤70 years, ≤pT2, ER+, Events ≥ 10% | 52 | |
| **TNBC** | TNBC NST | NST, ≤70 years, **ER/PR/Her2-**, pT≤ 2, G3, Events ≥ 10% | 100 | 400 |
| | TNBC NST | NST, ≤70 years, **ER/PR/Her2-**, Events ≥ 10% | 300 | |
| **Node-positives** | Affected lymph nodes, ER- | NST, ≤70 Jahre, ≥pT1c, N2a+, **ER-**, Events ≥ 10% | 34 | 99 |
| | Affected lymph nodes; ER+ | NST, ≤70 Jahre, ≥pT1c, N2a+, **ER+**, Events ≥ 10% | 65 | |

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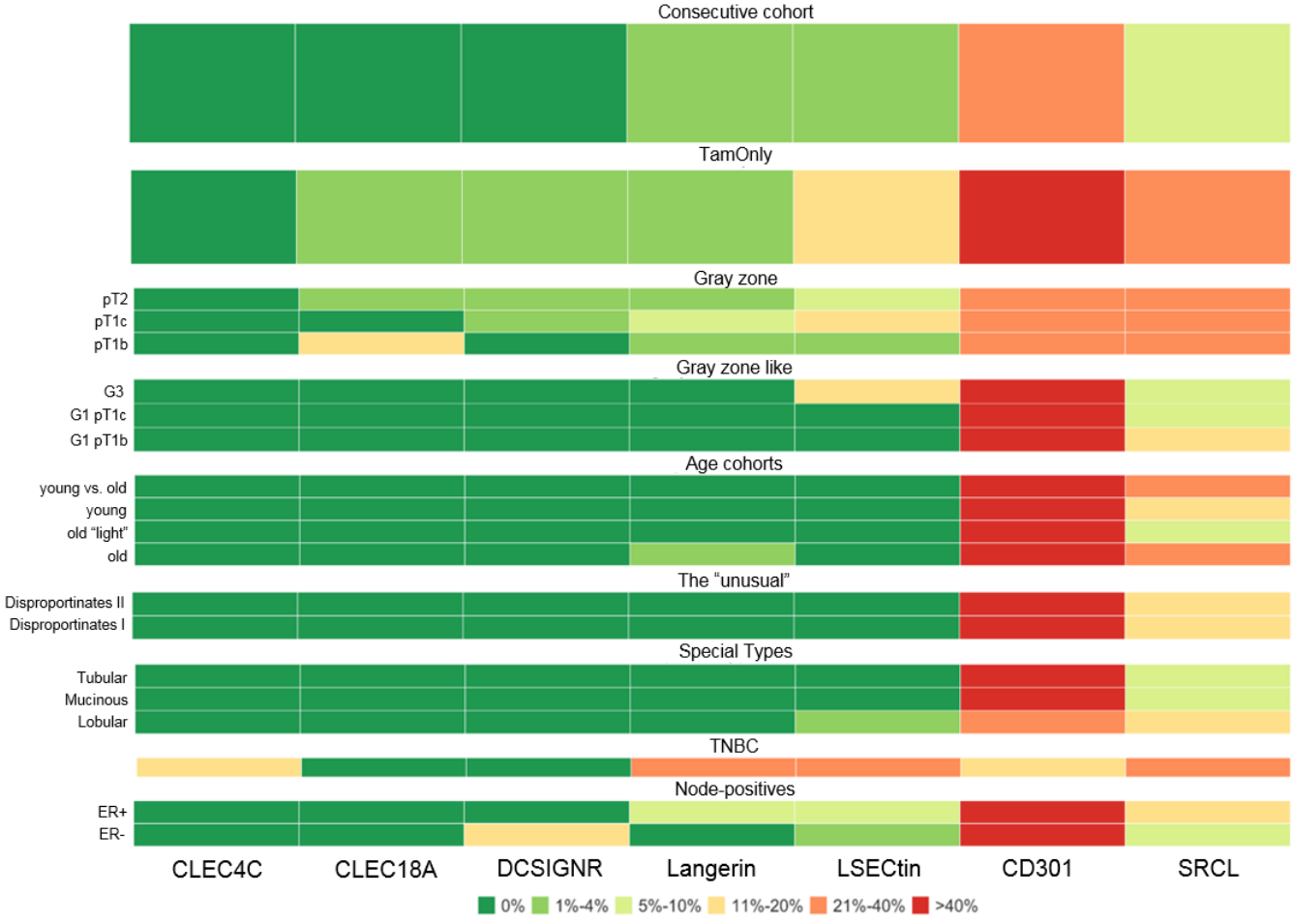


**Conclusions:** On the basis of this systematic approach we conclude that staining with GR in breast cancer can reveal clinically important additional information as far as typing and prognosis is concerned. The systematic concept of our TMA collection might enable a more focused approach in further studies to better understand the meaning of glycans in breast cancer.

## 235 Intraductal Papillomas Diagnosed in a Core Biopsy: Clinical, Radiological, and Pathologic Factors Associated with an Upgrade in Excisions

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**Disclosures:** Morad Qarmali: None; Jingjing Hu: None; Oluwole Fadare: None; Somaye Zare: None

**Background:** Intraductal papillomas (IDP) of the breast diagnosed in a core biopsy are often managed by surgical excision due to the possibility of an associated high risk lesion. Published data report a wide range of upgrade rates. We evaluated a variety of clinical, radiologic, and histopathologic features in IDPs to identify the features that are associated with an "upgrade" to a high risk lesion on the subsequent excision.

**Design:** The study set included consecutive cases of IDP diagnosed in a breast core needle biopsy and which were subsequently excised. Patients with concurrently diagnosed atypia or carcinoma, or a history of ipsilateral breast carcinoma were excluded. Clinical, pathologic, and radiologic data were reviewed and H&E slides were evaluated in a subset of cases with available pathology material. "High risk lesion" was defined broadly and included invasive and in situ carcinomas as well as atypias.

**Results:** A total of 219 cases met the study inclusion criteria, 31 (14.15%) of which showed an upgrade to a high-risk lesion on the follow-up resection. The high-risk lesions included 11 ductal carcinoma in situ (DCIS), 1 invasive ductal carcinoma, 11 atypical ductal hyperplasia, 1 flat epithelial atypia, and 7 lobular neoplasia. Patients that were upgraded to a high risk lesion on their resections were on average, significantly older than their counterparts without an upgrade (mean ages 59 versus 51 ($p<0.0001$). A history of carcinoma or atypia in the

contralateral breast was significantly associated with an upgrade in the resection. Among the patients with history of breast carcinoma or atypia, the rate of upgrade to a high-risk lesion was 25.8%. Other clinical and radiologic parameters did not show a significant risk for upgrade to a high risk lesion (table 1). A variety of pathologic features, including the length of longest contiguous focus of IDP, number and percentage of cores involved, necrosis, and calcifications were assessed in 128 patients with available slides. None of these pathologic features were associated with an upgrade on resection

Table 1:

| | **No high risk lesion on resection** | **high risk lesion on resection** | **P value** |
|---|---|---|---|
| **Number** | 188 | 31 | |
| **Age mean (range)** | 51 (22-76) | 59 (37-83) | p<0.0001 |
| **Imaging and clinical presentation** | | | Non-significant |
| Mass on imaging | 132 | 22 | |
| Calcification on imaging | 28 | 4 | |
| Discharge only | 18 | 4 | |
| Asymmetry or non-mass enhancement | 10 | 1 | |
| **Size on imaging** | | | Non-significant |
| <1 cm | 88 | 13 | |
| >1cm | 44 | 9 | |
| **History of cancer/atypia in contralateral breast** | | | p=0.0478 |
| Present | 23 | 8 | |
| Absent | 165 | 22 | |
| **BI-RADS score** | | | Non-significant |
| <4 | 48 | 7 | |
| 4 | 140 | 24 | |

**Conclusions:** Up to 14.4% of IDP diagnosed on core needle biopsies were upgraded to a high-risk lesion on resections, and 5.5% were associated with an invasive carcinoma or DCIS. None of the assessed radiologic or pathologic findings were significantly predictive of an upgrade. Older age and a past medical history of carcinoma/atypia in the contralateral breast were the only significant predictors of a high risk lesion on resection.

## 236 Expression of GATA3 and SOX10 in Invasive Mammary Carcinoma

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**Disclosures:** Muhammad Qazi: None; Stephanie McGregor: None

**Background:** Immunohistochemistry is the mainstay of evaluation for metastatic carcinoma of unknown primary, but markers that firmly establish mammary origin remain limited. The GATA3 and SOX10 transcription factors have employed in recent years toward this purpose, with the latter having broader coverage of triple negative breast cancers (TNBC), but their utility in combination has not been thoroughly explored.

**Design:** A preexisting tissue microarray produced from formalin-fixed, paraffin-embedded breast cancer cases with deidentified patient data, including hormone receptor status, was stained for SOX10 and GATA3. Hormone receptor (ER/PR) positivity was defined as $\geq$1%. Following exclusion of cases without represented invasive carcinoma, there remained 257 cases of invasive carcinoma (188 invasive ductal, 24 invasive lobular, 9 mixed ductal/lobular, and 22 other subtypes. Both stains were evaluated for the extent of tumor cells staining (<1%=negative, 1-10%, 11-50%, or >50%) and for intensity of the stain (GATA3 as weak, moderate, or strong; SOX10 as weak or strong). The proportion of positive cases was determined in relation to histologic subtype and receptor status.

**Results:** Among all cases combined, 92% were GATA3+ and 21% were SOX10+. Less than 1% of invasive mammary carcinoma cases were negative for both GATA3 and SOX10. A breakdown of combined GATA3 and SOX10 status is shown according to receptor status in the accompanying table (HR=hormone receptor).

| | HR+/HER2- | HR+/HER2+ | HR-/HER2+ | HR-/HER2- | TOTAL |
|---|---|---|---|---|---|
| GATA3+ SOX10- | 161 (87.0%) | 25 (89.3%) | 6 (54.5%) | 8 (24.2%) | 200 (77.8%) |
| GATA3+ SOX10+ | 21 (11.4%) | 1 (3.6%) | 4 (36.4%) | 11 (33.3%) | 37 (14.4%) |
| GATA3- SOX10+ | 3 (1.6%) | 2 (7.1%) | 0 (0.0%) | 13 (39.4%) | 18 (7.0%) |
| GATA3- SOX10- | 0 (0.0%) | 0 (0.0%) | 1 (9.1%) | 1 (3.0%) | 2 (0.8%) |
| TOTAL | 185 | 28 | 11 | 33 | 257 |

**Conclusions:** When used individually, GATA3 and SOX10 detect 92% and 21% of invasive mammary carcinoma and 58% and 73% of triple negative breast cancers, respectively. We also found that less than 1% of invasive mammary carcinoma are negative for both markers. Therefore, the combined use of GATA3 and SOX10 adds additional utility over using each marker individually in identifying mammary carcinoma. Care must be taken to use these markers in a panel that addresses other diagnostic considerations, such as other carcinomas and melanoma, particularly in the context of small biopsies. Our familiarity with these transcription factors is still evolving and further studies will elaborate their utility in establishing mammary origin.

## 237 Interobserver Variability in Breast Carcinoma Grading Results in Prognostic Stage Differences

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**Disclosures:** Kimmie Rabe: None; Veerle Bossuyt: None; Romulo Celli: None; Malini Harigopal: None; Olivia Snir: None; Emily Reisenbichler: None

**Background:** The 8th Edition of the AJCC Cancer Staging Manual incorporates tumor biomarker information, including histologic grade, into the pathological prognostic staging of breast cancer. Assessment of histologic grade gives valuable information about prognosis, however concerns over grading inconsistency between observers was a specific reason cited for its omission as a staging component in the manual's 7th edition. In this study, we aimed to assess the degree of interobserver agreement for tumor grade amongst pathologists and how variability in grading may affect the prognostic stage.

**Design:** A retrospective review of the electronic pathology database was performed to identify 100 sequential Stage II invasive breast carcinomas. A representative slide containing the largest tumor section was selected from each and pathologic data, including hormone receptor and HER2 status, tumor size and lymph node involvement recorded. Cases were independently reviewed by 6 pathologists and assigned Nottingham combined histologic grades (low, intermediate, high) based on established scoring for tubule formation, nuclear pleomorphism and calibrated mitotic count. Cases were graded again by the same 6 observers following a minimum 4-week washout period, this time with tumor receptor status provided. Cohen's k statistic was calculated using Vassarstat.net for intra- and inter-observer pairs. Fleiss' k for overall agreement amongst all observers was calculated in Microsoft Excel.

**Results:** 55 of 100 cases were classified as invasive ductal (no special type), 36 lobular or ductal with lobular features, and 9 other special types. Intra-observer agreement ranged from k of 0.71 to 0.83 (mean=0.77). For observer pairs on the first review, agreement ranged from k of 0.59 to 0.85 (mean=0.72, Table 1). For the second review, observer pair agreements were similar, ranging from k of 0.58 to 0.86 (mean=0.72, data not shown). Round one grading resulted in an overall k of 0.675 with the same grade assigned by all 6 observers in 54 cases. In the 46 cases with discordant grading, 22 resulted in the assignment of a different pathological prognostic stage. The most frequent stage discordance, in 14 cases, was between prognostic stages IB and IIA.

Table: Pairwise Kappa Agreement (standard error) for Invasive Carcinoma Histologic Grading

| | **Pathologist 2** | **Pathologist 3** | **Pathologist 4** | **Pathologist 5** | **Pathologist 6** |
|---|---|---|---|---|---|
| **Pathologist 1** | 0.726 (0.057) | 0.829 (0.047) | 0.772 (0.054) | 0.645 (0.065) | 0.743 (0.056) |
| **Pathologist 2** | | 0.845 (0.044) | 0.754 (0.057) | 0.629 (0.068) | 0.730 (0.057) |
| **Pathologist 3** | | | 0.778 (0.056) | 0.709 (0.059) | 0.734 (0.059) |
| **Pathologist 4** | | | | 0.607 (0.069) | 0.716 (0.057) |
| **Pathologist 5** | | | | | 0.594 (0.068) |

**Conclusions:** Despite moderate overall inter-observer agreement in tumor grading by statistical standards, discordant grading resulted in different prognostic stages in 22% of cases. This raises concern regarding the reliability of incorporating tumor grade into breast cancer staging.

## 238 Analysis of Genetic Mutations in Sporadic and BRCA1 Carrier Breast Cancers by Next Generation Sequencing

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**Disclosures:** Cynthia Reyes Barron: None; Andrew Campbell: None; Paul Rothberg: None; David Hicks: None; Xi Wang: None

**Background:** BRCA1 mutant carriers have a lifetime breast cancer risk up to 80%. Studies have shown that TP53 mutations may be a necessary step in carcinogenesis of BRCA1 carriers.

**Design:** Next generation sequencing (NGS) testing was performed on 35 breast carcinoma (BC) cases, including six BRCA1 carriers (one with bilateral BC) and 29 sporadic BCs, using the Oncomine Comprehensive Assay by Thermo Fisher Scientific on an Ion Torrent S5 XL sequencer. Single nucleotide variants (SNVs) with a variant allele frequency <5% and/or coverage with <400 reads were excluded. Pathogenicity was determined using ClinVar and Varsome websites.

**Results:** The bilateral BCs in a BRCA1 carrier showed different morphology and ER/PR/Her2 status: triple negative (TN) versus ER+/PR+/Her2-. Both tumors had alterations in TP53 and NOTCH3: TN tumor with TP53 frameshift and NOTCH3 missense (uncertain significance), and ER+/PR+/Her2- tumor with TP53 pathogenic missense and NOTCH3 frameshift. In addition, they shared 6 SNVs of uncertain significance (indicating germline pleomorphism). Only one different SNV of uncertain significance was identified between them. Of the remaining four BRCA1 associated BCs, two of the TNBC had TP53 missense mutations but no other known pathogenic mutations. One TNBC and one ER+/PR+/Her2- tumor did not have any known pathogenic mutations.

Eight sporadic TNBCs had TP53 mutations; of these, five had one (4) or two (1) additional known pathogenic mutations, including NOTCH3 (1), PTEN (1), MSH2 (1), SLX4 (1), AKT1 (1), BREBBP (1). Seven sporadic TNBCs were TP53 negative; of these, six had one (4) or two (2) known pathogenic mutations, including PIK3CA (2), PTEN (1), NF1 (2), BRAF (1), CREBBP (1), PALB2 (1).

One of six sporadic ER+/PR+/Her2-/p53+ cases had over 80 SNVs, and five were known pathogenic (PTEN, NF1, SMARCA4, CREBBP, TSC2). Of the remaining five, only one had an additional known pathogenic mutation (NF1). Only two of eight sporadic ER+/PR+/Her2-/p53- BCs had known pathogenic mutations: PIK3CA (1), PTEN (1), ATM (1).

| | **BRCA1 Carrier Breast Cancers** | | | **Sporadic Breast Cancers** | | |
|---|---|---|---|---|---|---|
| **TP53** | Triple Negative | ER+/PR+/Her2- | Total | Triple Negative | ER+/PR+/Her2- | Total |
| *mutated* | 3 | 1 | 4 | 8 | 6 | 14 |
| *wild type* | 1 | 1 | 2 | 7 | 8 | 15 |

**Conclusions:** Known pathogenic gene mutations are not common and with very low recurrence rate in BCs, including BRCA1 associated BCs. TP53 mutations do not increase the number of pathogenic SNVs in BCs, including in BRCA1 associated BCs. A TP53 mutation may not be a necessary step in carcinogenesis of BRCA1 associated BCs.

## 239 Detection of ERBB2 Copy Number Variants by Next Generation Sequencing and Comparison to Amplification Detection by Fluorescence in Situ Hybridization, Protein Quantitation Using Fluorescent Nanoparticles and Immunohistochemistry

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**Disclosures:** Cynthia Reyes Barron: None; Andrew Campbell: None; Brandon Buscaglia: None; Xi Wang: None; David Hicks: None; Yi Ding: None

**Background:** Over-expression of Human Epidermal growth factor Receptor-2 (HER2)/Erb-B2 receptor tyrosine kinase 2 (*ERBB2*) in breast cancer is associated with an aggressive clinical course and benefit from targeted therapy. Current clinical assays include immunohistochemistry (IHC) to detect protein over-expression and fluorescence in situ hybridization (FISH) to detect gene amplification. HER2 may be quantitatively measured using streptavidin-coated Phosphor Integrated Dot fluorescent nanoparticles (PID) with immunofluorescence. IHC, FISH, and PID have limitations and may have discordant results. Our aim was to compare the results of next generation sequencing (NGS) with these methods.

**Design:** Thirty-five cases of breast invasive ductal carcinoma, which had previously undergone IHC, FISH, and PID testing, were selected. NGS study was performed using the Oncomine Comprehensive Assay on an Ion Torrent S5 XL sequencer. HER2 amplification was considered positive by IHC with value 3+, PID with value ?30/cell and NGS with copy number (CN) ?5. Results were compared. Single nucleotide variants (SNVs) in ERBB2 with variant allele frequency >5% and coverage >400 reads were considered. Pathogenicity was determined using ClinVar and Varsome.

**Results:** Four cases were HER2 amplified by NGS (CN 8.15 to16.92). All four were classical amplified by FISH; 3 had IHC score 3+ and 1 of 2+; all were positive by PID (155.5 to 343.5). One case was classical amplified by FISH, had IHC 3+, PID 148.1 and CN 2.94 by NGS (negative). Of the remaining 30 cases with FISH results: low level amplified (9), monosomy (5), polysomy (4), equivocal (5) and non-amplified (7); all had IHC 0 to 2+, PID score 2.6 to 22.5, and CN 1.12 to 4.18 by NGS (see table). There was no correlation between CN determined by NGS and PID score.

Eleven cases had a missense SNV in ERBB2, 3 had two missense SNVs; 6 were variants of undetermined significance and 11 were benign. The benign p.Ile655Val SNV was identified in 10 cases with variant allele frequency 8.15% to 99.8%. There was no correlation between presence of SNVs and ERBB2 amplification.

| **FISH Category** | **Classical Amplified** | **Low-level Amplified** | **Monosomy Ratio Positive** | **Polysomy Ratio Negative** | **Equivocal** | **Classical Non-Amplified** |
|---|---|---|---|---|---|---|
| | ratio ?2, HER2 ?6 | ratio ?2 HER2 ?4 <6 | ratio?2 HER2 <4 | ratio <2 HER2 ?6 | ratio <2 HER2 ?4 <6 | ratio <2 HER2 <4 |
| ***Total cases*** | **5** | **9** | **5** | **4** | **5** | **7** |
| NGS + | 4 | 0 | 0 | 0 | 0 | 0 |
| PID + | 5 | 0 | 0 | 0 | 0 | 0 |
| IHC + | 4 | 0 | 0 | 0 | 0 | 0 |

**Conclusions:** NGS provided highly concordant results (80%) with established methods of HER2 amplification for classic amplified cases by FISH. Other FISH results were negative by NGS, IHC and PID. The potential for NGS in classifying the nature of ERBB2 genetic alterations remains to be explored and may help further elucidate cases with non-classical FISH results or incongruous protein over-expression by PID.

## 240 Molecular Profiles of Lung Metastases in Breast Cancer

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**Disclosures:** Travis Rice-Stitt: None; Melissa Krystel-Whittemore: None; Valentina Nardi: None; Mari Mino-Kenudson: *Consultant*, Merrimack Pharmaceuticals; *Consultant*, H3 Biomedicine; Elena Brachtel: None

**Background:** Molecular alterations specific to lung metastases of breast cancer have not been investigated in detail. Prior studies suggest enrichment of basal-like breast cancers metastatic to lung, but the molecular associations need further evaluation. Mutations of known significance in breast cancer, including PIK3CA and TP53, also need characterization in lung metastases.

**Design:** The study cohort consists of 46 patients with lung metastases from primary breast cancer. The metastatic lung samples were tested for oncogenic mutations from 2013-18 using SNAPSHOT, an Anchored Multiplex PCR assay which detects single nucleotide variants and insertions/deletions using the ArcherDx and Illumina NextSeq NGS platforms. The samples were collected by needle biopsy (n=16), surgical resection (n=10), or thoracentesis of exfoliated cells in pleural fluid (n=16). Available clinicopathologic characteristics and ER and HER2 testing (IHC and FISH) were evaluated in lung metastasis (n=41) and breast primaries (n=37).

**Results:** 61 oncogenic mutations were found in 29 patients. 15 patients had no reportable mutations and 2 biopsy specimens failed testing due to insufficient DNA. The most frequent mutations were in TP53 (n=14, 30%) and PIK3CA (n=10, 22%). Less frequent mutations involved CDH1 (n=3), BRCA1/2 (n=3), IDH1/2 (n=2), PIK3R1 (n=2), and ESR (n=2). Primary breast cancer occurred at a mean age of 52 years (range 27-82) and lung metastases at a mean age of 60 years (range 36-90). Primaries were grade 2 (n=15) or 3 (n=23), with a mean tumor size of 2.3 cm, and either invasive ductal (n=31), invasive lobular (n=2), invasive carcinoma with mixed features (n=4), or invasive metaplastic carcinoma (n=2). 56% had lymph node involvement (N1-N3). Most lung metastases were ER positive (59%, n=24), 6 were HER2 amplified (15%), and 15 were triple negative (37%). The average interval between primary diagnosis and metastasis was 8.4 years, with 10.2 years in ER positive (n=21) and 6.4 years in ER negative metastases (n=19).

| Mutation | Frequency | ER+ | HER2+ | Triple negative | Interval between primary and metastasis (years) |
|---|---|---|---|---|---|
| No Variants | 15 | 3 | 2 | 7 | 10 |
| TP53 | 11 | 5 | 4 | 5 | 5 |
| PIK3CA | 7 | 6 | 0 | 1 | 11 |
| TP53 & PIK3CA | 3 | 1 | 0 | 1 | 7 |
| CDH1 | 3 | 3 | 0 | 0 | 12 |
| BRCA1/2 | 3 | 1 | 0 | 2 | 4 |
| IDH1/2 | 2 | 2 | 1 | 0 | 8 |
| PIK3R1 | 2 | 2 | 2 | 0 | 4 |
| ESR | 2 | 2 | 0 | 0 | 13 |

**Table 1.** Mutations detected in at least 2 cases are listed above. Mutations found in only 1 case (not reported above) include: AKT1, EGFR, NOTCH1, MAP3K1, SMARCA4, FGFR1, ARIDIA, FOXL2, CDKN2A, NF1, ERBB2, and RET.

**Conclusions:** Lung metastases were disproportionally triple negative and commonly harbored TP53 and PIK3CA mutations. TP53 mutations were associated with a short interval between primary and metastasis and PIK3CA was associated with a longer interval (p=0.012). Cases with no mutations were associated with a long interval, despite a high proportion being triple negative. Pleural fluid specimens offer adequate DNA for testing. Mutations were identified in multiple genes associated with basal-like breast cancer.

## 241 Triple Negative Breast Cancers (TNBC) with Luminal Features: Categorization Based on Androgen Receptor (AR), Retinoblastoma (Rb) Protein, and p53 Expression and Implications for Treatment

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**Disclosures:** Edward Richardson: None; Vanda Torous: None; Jaymin Patel: None; Andrew Goss: None; Michele Hacker: None; Nadine Tung: None; Stuart Schnitt: None

**Background:** TNBC, by definition, lack estrogen receptor (ER) and progesterone receptor expression and are HER2 negative. As a group, these tumors are characterized by a high level of genomic instability, have frequent *TP53* mutations, and have a poor prognosis. The most appropriate systemic therapy remains an area of active investigation. While most TNBC have a basal phenotype, a subset shows expression of AR and active signaling through the AR pathway. Such tumors are now considered the luminal AR (LAR) subtype of TNBC. Ongoing research is assessing whether LAR TNBC have sufficient luminal-type biology to benefit from treatments now used for patients with ER-positive (luminal) tumors. For example, the majority of ER-positive breast cancers are Rb proficient, which in turn renders them sensitive to CDK4/6 inhibitors. TNBC with an intact Rb pathway may similarly benefit from these drugs. Therefore, an understanding of Rb status of TNBC in relation to AR and p53 expression could help determine which TNBC are more likely to have luminal-like biology and the potential for similar responses to treatment.

**Design:** Sections cut from tissue microarrays of 180 TNBC were immunostained for a broad panel of biomarkers including high and low molecular weight cytokeratins, AR, Rb protein and p53. Expression of Rb and p53 were used to further characterize TNBC that were AR-positive.

**Results:** Among the 180 TNBC, 12.2% were AR-positive, 51% were Rb-positive, and 58% were p53-positive. TNBC that were AR-positive were significantly more often Rb-positive than those that were AR-negative (77% vs 47%; p=0.008). AR-positive cancers and AR-negative cancers had a similar frequency of p53 positivity (67% vs 57%, p=0.21). However, TNBC that were negative for both AR and Rb were more likely to be p53 positive (implying p53 mutation) than TNBC that are positive for both AR and Rb, and this difference approached but did not reach statistical significance (69% vs 55%; p=0.08).

**Conclusions:** Our results suggest that combined evaluation of AR, Rb and p53 provides additional insights into TNBC biology with therapeutic implications. TNBC showing expression of both AR and Rb are less often p53-positive than TNBC that lack expression of both AR and Rb. This observation raises the possibility that AR-positive/Rb-positive/p53-negative TNBC may have luminal-like biology and, therefore, benefit from treatments that are used for patients with ER-positive (luminal) breast cancers such as CDK4/6 inhibitors.

## 242 Analysis of Concordance Between Radiologic Imaging Modalities, Final Pathologic Tumor Size and Focality in Invasive Breast Carcinoma

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**Disclosures:** Marilin Rosa: None; Emmanuel Agosto-Arroyo: None; Laila Khazai: None; Lorena Di Pasquale Guadalupe: None; Jose De Jesus: None; Yin Xiong: None

**Background:** Accurate assessment of pathologic (pT) and clinical tumor size (cT) are crucial for patient treatment and prognosis. For cT, radiological findings carry a significant weight. The aim of this study was to evaluate the correlation of the tumor size and focality between radiologic studies (mammogram [MG], ultrasound [US] and magnetic resonance imaging [MRI]) and final pathology and to assess possible effects on patient staging.

**Design:** Our information systems, Pathnet and PowerChart were searched to identify all patients who underwent excisional procedures for invasive breast carcinoma from January 1, 2014 to December 31, 2015.

**Results:** From the 1380 primary breast excisions/mastectomies performed during the study period, 490 cases were included. Of those, 402 were invasive ductal carcinomas (IDC), 56 were invasive lobular carcinomas (ILC), and 32 cases were special type carcinomas. 27 cases had an extensive intraductal component (EIC). 1,019 preoperative radiologic studies were evaluated (412 US, 335 MG and 272 MRI). There was a statistically significant difference between the mean size of the tumor on the final pathology report (mean 1.73 cm) and the mean size on radiologic imaging, being 2.14 cm (p-value: 0.0000232) on MG, 2.14 cm (p-value: 0.0039) on US and 2.87 cm respectively (p-value: <0.0001) on MRI. In 296 studies (29%), there was a discrepancy in tumor stage. In comparison with pT stage, MRI had the highest error rate, 89 of 272 studies (33%, 77 cases overstaged and 12 understaged) and MG was second with 106 of 335 studies (32%, 73 cases overstaged and 33 cases understaged). The modality with the least error rate was US, 101 of 412 studies (25%) with 63 cases understaged and 38 overstaged. An EIC component was the variable most closely linked to discrepancies between cT and pT stage, with overstaging in 14 out of 22 cases on MG (63.64%), 13 out of 19 on MRI (68.42%) and 8 out of 11 cases on US (73%). The error rate in number of foci identified was 28% on MG, 27% on US and 29% on MRI with tendency to underestimate foci on MG and US, and overestimate foci on MRI.

**Conclusions:** Our study revealed that there are statistically significant differences in mean tumor size and multifocality across the three imaging modalities when compared to the final pathology. MRI had the highest error rate (33% for size and 29% for focality) with a tendency to overestimate. Among all diagnoses, cases of invasive carcinoma with EIC were more prone to discrepancy with imaging.

## 243 Potential Biomarkers of Immunotherapy Responsiveness in Triple Negative Breast Cancer

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**Background:** Triple negative breast cancer (TNBC) is an aggressive form of the disease that has been difficult to treat after relapse or metastasis occurs post-adjuvant chemotherapy. We queried whether comprehensive genomic profiling (CGP) of TNBC could identify biomarkers that have been linked to responsiveness to immune check point inhibitor (ICPI) treatments and assist in the selection of immunotherapy approaches for these patients.

**Design:** DNA was extracted from 40 microns of FFPE specimen from 643 cases of relapsed and metastatic TNBC. CGP was performed using a hybrid-capture, adaptor ligation based next generation sequencing assay to a mean coverage depth of >500X. Tumor mutational burden (TMB) was determined on 1.1 Mbp of sequenced DNA and microsatellite instability (MSI) was determined on 114 loci in 564 cases. PD-L1 was determined by IHC on 68 cases.

**Results:** The women in this study had a median age of 53 years (range 20-85 years). All TNBC were negative for ER and PR by IHC and for ERBB2 amplification by CGP. The primary breast tumor was used for sequencing in 56% of cases and a local recurrence or metastasis biopsy in 44% of cases. There were 5.9 GA/tumor. The median TMB was 3.5 mutations/MB. Most frequent GA were TP53 (88%) and MYC (28%). Potential MTOR pathway targets included *PIK3CA* (19%), *PTEN* (15%), and *NF1* (8%). Kinase targets included *FGFR1* (8%), *FGFR2* (4%), *KIT* (2%) and *MET* (1%). *BRCA1* was altered in 7% and *BRCA2* in 3% of cases. *AR* was amplified in

1%. Markers of potential ICPI benefit included CD274 (PD-L1) amplification (3%), *BRAF* GA (4%), TMB of ≥10 mut/Mb (9%), TMB of ≥20 mut/Mb (2%), MSI-High (0.4%), *PBRM1* GA (1%) and low (10%) or high (3%) positive PD-L1 staining. Potential markers of resistance included inactivating mutations in *STK11* (2%) and *MDM2* amplification (3%). Examples of TNBC patients responding to ICPI therapies will be presented.

**Conclusions:** : In addition to guiding targeted therapy selection, CGP also shows significant potential to characterize the GA that have been linked to response and resistance to ICPI in TNBC. As also shown by clinical examples in this study, and reported previously, the benefit of immunotherapy in metastatic TNBC supports the need for the development of biomarkers used to guide the use of ICPI drugs for these patients.

## 244 High Copy Number and Expression of RECQL4 and SDHC In Breast Tumors of Ghana Patients

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**Disclosures:** Miguel Rufail: None; Talha Anwar: None; Lisa Newman: None; Scott Tomlins: *Grant or Research Support*, ThermoFisher Scientific; *Employee*, Strata Oncology; *Major Shareholder*, Strata Oncology; Celina Kleer: None

**Background:** Breast cancer in African women and in African Americans has a higher incidence of triple negative, high grade aggressive subtypes. The reasons are not fully understood. The role of the RECQL4 and SDHC genes in breast cancer has not been studied in depth so far. RECQL4 is a helicase involved in DNA replication that regulates genomic stability. Alterations of this gene have been found in Rothmund-Thomson syndrome, which carries high risk of several malignancies. Succinate dehydrogenase C (SDHC) is involved in aerobic metabolism. SDHC alterations have been mostly found in gastrointestinal stromal tumors, pheochromocytomas, and renal carcinoma. In the present study, we set out to discover the most frequent gene copy number alterations in a cohort of invasive carcinomas from Ghanaian women.

**Design:** Representative breast carcinomas from patients from Ghana (n=11) were analyzed by targeted next-generation sequencing (NGS) to identify somatic alterations. We histologically characterized and constructed a high density tissue microarray (TMA) with 86 invasive carcinomas from Ghana. We assessed the expression of RECQL4 and SDHC proteins by immunohistochemistry in the TMAs. Breast cancer TCGA dataset was analyzed using UALCAN and KM plot.

**Results:** NGS revealed copy number variations in all tumors, involving 17 genes. High level copy number of RECQL4 and SDHC were the most common alterations, each present in 6 of 11 tumors (54.5%), occurring together in 4 cases (36.4%). TMA immunohistochemistry showed that 62% of Ghanaian invasive carcinomas are positive for RECQL4 and 56% for SDHC. In TCGA dataset, expression of RECQL4 and SDHC is significantly higher in invasive carcinomas compared to normal breast ($p<0.001$). RECQL4 expression is significantly higher in tumors from African-American women compared to Caucasians ($p<0.001$). Triple negative breast carcinomas with high expression of RECQL4 or SDHC have a worse disease free survival than those with low expression (log rank $p<0.001$, and $p=0.004$, respectively).

**Conclusions:** Our study revealed that invasive carcinomas from African women have increased copy number alterations of RECQL4 and SDHC, which was previously unknown. We validated the increased expression of RECQL4 and SDHC proteins in a large unique cohort of Ghanaian breast carcinomas, and breast cancer TCGA datasets. These data shed light into the genetic abnormalities of aggressive invasive carcinoma in African patients and may have prognostic and/or therapeutic value.

## 245 Core Needle Biopsy Diagnosis of Fibroepithelial Lesions: Features Predictive of Upgrade to Phyllodes Tumor at Excision

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**Disclosures:** Elena Salagean: None; Katia Ventura: None; Edi Brogi: None; Melissa Murray: None

**Background:** Fibroepithelial lesions (FELs) of the breast are a heterogenous group of neoplasms encompassing fibroadenoma (FA) and phyllodes tumor (PT). Morphologic overlap between cellular FA and PT limits their definitive diagnosis on review of core needle biopsy (CNB) material. However, FAs are not necessarily excised, whereas surgical excision is recommended for PTs, that also have higher risk of local recurrence. We sought to assess histologic features of FELs diagnosed at CNB associated with an upgrade to PT at excision (EXC).

**Design:** A search of our institutional database identified inhouse CNBs with diagnosis of FEL rendered between 2000 to 2018. CNBs with no follow-up EXC were excluded. The CNB slides were re-reviewed. The morphologic features of FELs were recorded, including stromal cellularity and overgrowth (4x and 10x magnification), fragmentation, lesion circumscription/infiltration, heterogeneity, subepithelial condensation, stromal cell nuclear pleomorphism, stromal vascularity, mitoses/10 high power fields, and pseudoangiomatous hyperplasia

(PASH). Two-tailed Student's t-test was done to identify statistically significant differences between means and the Chi Square test was used for categorical variables.

**Results:** We identified 60 CNBs from 58 patients (2 patients had bilateral CNBs) with EXC and with a median age of 39 (range 14 to 77 years old). Twenty-eight CNBs (46.7%) yielded PT at EXC, including 24 (85.7%) benign PTs, 3 (10.7%) borderline PTs, and one tumor with features straddling between benign and borderline PT (3.6%). Our findings are summarized in table 1.

**Table 1:** Histopathologic Features of FELs diagnosed on CNB

| Features Analyzed | FEL Upgraded N=28 | % upgraded | FEL Not Upgraded N=32 | % not upgraded | p value |
|---|---|---|---|---|---|
| **Average age (years)** | **42.8** | | **35.3** | | **0.0388** |
| **Stromal cellularity:** | | | | | |
| High | 6 | 10.0% | 4 | 6.5% | 0.3545 |
| Intermediate | 21 | 35.0% | 23 | 37.7% | 0.7848 |
| Low | 1 | 1.7% | 5 | 8.2% | 0.1205 |
| PASH | 9 | 15.0% | 10 | 16.4% | 0.9409 |
| **Stromal cell nuclear pleomorphism** | **28** | **46.7%** | **21** | **34.4%** | **0.0024** |
| **Mitoses >=1** | **18** | **30.0%** | **10** | **16.4%** | **0.0105** |
| Infiltrative borders | 3 | 5.0% | 7 | 11.5% | 1.3393 |
| **Heterogeneity** | **24** | **40.0%** | **19** | **31.1%** | **0.0239** |
| Fragmentation | 11 | 18.3% | 9 | 14.7% | 0.3602 |
| Stromal Vascularity | 13 | 21.7% | 9 | 14.7% | 0.1422 |
| Subepithelial condensation | 10 | 16.7% | 14 | 22.9% | 0.5262 |
| Overgrowth (4x magnification) | 0 | 0% | 0 | 0% | n/a |
| Overgrowth (10x magnification) | 5 | 8.3% | 3 | 4.9% | 0.3349 |

**Conclusions:** Surgical excision of FELs yields an upgraded diagnosis of PT in 46.7% of cases. The presence of mitoses, stromal cell nuclear pleomorphism, lesion heterogeneity and patient age may help predict upstaging of FEL to PT.

## 246 Atypical Ductal Hyperplasia (ADH) in Breast Core Needle Biopsies (CNB): Is Excision Always Warranted? Review of The Experience at a Tertiary Care Center

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**Disclosures:** Elena Salagean: None; Anne Grabenstetter: None; Sandra Brennan: *Consultant*, Intrinsic imaging; Edi Brogi: None

**Background:** The CNB diagnosis of ADH mandates follow-up surgical excision (EXC), but controversy exists whether minute foci of ADH (fADH) should be excised. We reviewed our experience to address this question and identify features of ADH that correlate with upgrade at EXC.

**Design:** We searched the pathology database to identify consecutive inhouse CNBs with diagnosis of ADH obtained between 2015-2017. We further included in the study 17 CNBs with original diagnosis of flat epithelial atypia (FEA) reclassified as fADH. We excluded CNBs without EXC. The histologic and imaging features were recorded and analyzed. A study radiologist re-reviewed all pertinent imaging studies. fADH was defined as a single focus of low grade ductal atypia spanning <2.0 mm. Upgrade was defined as invasive carcinoma (IC) or ductal carcinoma in situ (DCIS) in the EXC. All EXC slides with upgrade were re-reviewed.

**Results:** Our study cohort consists of 101 inhouse CNBs from 94 patients (pts), with median age 53 years (range 33-79). Five pts had bilateral CNBs, 2 ipsilateral CNBs. Thirty-five (35%) pts had prior/concurrent breast carcinoma (HxBC).

The CNB target was calcifications ($Ca^{2+}$) in 82 (81%) cases, a mass in 6 (6%), a mass with $Ca^{2+}$(1 case, 1%), and an MRI abnormality in 12 (12%).

A total of 22 (22%) CNBs from 20 pts were upgraded on EXC (9 IC, 12 DCIS). The CNBs with upgrade also had: 5 classic lobular neoplasia, 4 FEA, 4 focal necrotic debris, and 2 apoptosis. The upgrade rate in pts with HxBC was 12/35 (34%). On re-review, 15/101 (15%) CNBs from 15 pts (including 6 pts with HxBC), had fADH (Table 1). Three of 15 (20%) CNBs with fADH from 3 pts yielded an upgrade: 2 ICs (2 mm ductal, 2.1 cm lobular) and 1 DCIS (4 mm low-intermediate nuclear grade). Two of these 3 pts had HxBC. The CNB

of the single pt with upgrade and no HxBC had fADH (1.8 mm span) with apoptosis and FEA; the corresponding upgrade was a 2 mm IC well differentiated, not adjacent to the biopsy site (query incidental finding).

**Table 1:** Clinico-pathologic features of core needle biopsies with focal ADH.

| Case | Age | Imaging Modality | Imaging Review | Target Removed with CNB | Prior/Concurrent History of Breast Carcinoma | CNB Findings | Excision Findings |
|---|---|---|---|---|---|---|---|
| 1 | 52 | Mammography | 8 mm $Ca^{2+}$ | Yes | ILC | 1.2 mm ADH, ALH, LCIS | ILC, 2.1 cm |
| 2 | 47 | Mammography | 9 mm $Ca^{2+}$ | Yes | Absent | 1.8 mm ADH | IDC, 2 mm |
| 3 | 79 | Ultrasound | 4 mm mass | No | Concurrent ipsilateral IDC | 0.5 mm ADH | DCIS, 4 mm |
| 4 | 52 | Mammography | 3 mm $Ca^{2+}$ | No | Concurrent contralateral DCIS | 1.9 mm ADH | Benign breast with radial scars |
| 5 | 47 | Mammography | 4 mm Ca2+ | Yes | Prior contralateral DCIS | 0.2 mm ADH | Benign breast |
| 6 | 68 | Mammography | 3 mm Ca2+ | No | Concurrent contralateral IDC | 1.1 mm ADH | ADH |
| 7 | 63 | Mammography | 2 mm Ca2+ | No | Absent | 0.9 mm ADH | LCIS |
| 8 | 61 | Mammography | 6 mm Ca2+ | Yes | Prior ipsilateral IDC | 0.8 mm ADH | FEA |
| 9 | 47 | Mammography | 5 mm Ca2+ | No | Absent | 0.7 mm ADH | Benign breast |
| 10 | 49 | Mammography | 5 mm Ca2+ | No | Absent | 1.0 mm ADH | ADH |
| 11 | 59 | Mammography | 7 mm mass | Yes | Absent | 0.8 mm ADH | Benign breast |
| 12 | 7 | Mammography | 3 mm Ca2+ | Yes | Absent | 1.1 mm ADH | Benign breast |
| 13 | 54 | Mammography | 8 mm calcifications | Yes | Absent | 0.5 mm ADH | ADH |
| 14 | 50 | Mammography | 8 mm $Ca^{2+}$ | No | Absent | 1.3 mm ADH | Benign breast |
| 15 | 46 | MRI | 4 mm focus of enhancement | Yes | Absent | 0.1 mm ADH | Benign breast |

ILC = invasive lobular carcinoma, IDC = invasive ductal carcinoma, ALH = atypical lobular hyperplasia, LCIS = lobular carcinoma in situ

**Conclusions:** Our data shows an overall upgrade rate of 22% at excision of ADH, and up to 30% in patients with prior/concurrent breast carcinoma. The upgrade rate of focal ADH was 20% for all patients, but it was lower (1/9 patients) in the subgroup without prior/concurrent carcinoma. The single upgrade in our series consisted of a minute low grade carcinoma. Evaluation of more cases with detailed radiologic-pathologic correlation is in progress. This information could be used for management planning of patients with ADH.

## 247 Carbonic Anhydrase IX Identifies an Aggressive Subset of Triple Negative Breast Ductal Carcinomas in African American Women

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**Disclosures:** Daniel Sanchez: None; Abiye Kassa: None; Ali Afsari: None; Luisel Ricks-Santi: None; Tammey Naab: None; Ashwini Esnakula: None

**Background:** Hypoxia in tumors is associated with resistance to neoadjuvant chemotherapy and radiotherapy and a more aggressive phenotype. Carbonic anhydrase isoform IX (CAIX) expression is increased in response to hypoxia via hypoxia inducible factor (HIF-1) dependent mechanism, facilitating tumor progression. CAIX expression has been linked to adverse prognosis in hormone negative breast ductal carcinomas. We aimed to assess the prognostic significance of CAIX expression in subtypes of breast ductal carcinomas and correlate with clinicopathologic risk factors in African American (AA) women.

**Design:** Tissue microarrays were constructed from formalin fixed parffin embeded tumor blocks from primary ductal breast carcinomas in 236 AA women. Two separate 1 mm cores represented each case. Five µm sections were stained with a rabbit monoclonal antibody against CAIX (clone EP161, Cell Marque, CA, USA). The sections were assessed for intensity and extent of membranous staining. H score greater than or equal to 5 was considered positive. Bivariate analysis was done via chi-square analysis and survivability data was calculated via the generation of Kaplan-Meier curves (SPSS v19). Statistical significance was assumed if $p < 0.05$.

**Results:** CAIX expression was identified in 35 of 200 evaluated cases. High expression (H score >100) was noted in 10 cases. 27 of 94 (28.7%) triple negative breast ductal carcinomas (TNBCs) were positive for CAIX. CAIX expression was significantly associated with TNBCs ($p = 0.0001$), ER negative ($p<0.0001$), PR negative ($p<0.0001$), high grade ($p=0.002$), and high stage ($p= 0.012$) breast ductal carcinomas. CAIX expression was marginally associated with overall survival ($p=0.066$). CAIX expression was significantly associated with high stage ($p=0.031$) and metastases ($p=0.038$) in the TNBCs.

**Conclusions:** In our AA population, a statistically significant association was found between CAIX expression and high stage TNBCs. Hypoxic tumor cells express CAIX to maintain a physiologic intracellular pH and simultaneously contributing to an acidic extracellular pH, which leads to enhanced tumor cell survival. The association of CAIX with high stage TNBCs in AA women indicates that this isoform plays a role in progression of subset of these tumors. Isoform-specific targeting of CAIX activity by monoclonal antibodies or small molecules may represent a new targeted therapy option for TNBCs.

## 248 Pregnancy-Associated Breast Cancer: Clinicopathologic Differences in Patients Diagnosed during Pregnancy and in Postpartum Period

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**Background:** Pregnancy-associated breast cancer (PABC) is currently defined as breast cancer diagnosed during pregnancy or within 1 year of postpartum period. Existing literature is inconsistent in relation to its clinical definition, histologic characteristics, and pathophysiology. Limited data suggests that breast cancers developing during postpartum phase have adverse prognosis secondary to its unique involuting microenvironment. Herein we present our single institution experience with PABC, together with comparative analysis of breast cancers diagnosed during pregnancy with those diagnosed within 2 years postpartum.

**Design:** This retrospective study includes a cohort of PABC cases retrieved from our pathology database between 1990 and 2018. Cases were divided into 2 study groups: group 1 (diagnosed during pregnancy) and group 2 (diagnosed within 2 yrs of postpartum). Clinical and pathologic variables and survival data were compared and statistical analysis was done.

**Results:** We identified a total of 51 cases, 28 in group 1 and 23 in group 2. In group 2, 17 cases were diagnosed within 1 yr and 6 cases within 2 yrs of postpartum. Median age was 34 yrs (range, 24 to 43). The clinicopathologic characteristics of the two groups are summarized in table 1. Group 1 consisted of 14 luminal type, 4 HER2 positive, 6 triple negative (TN) and 4 triple positive (TN) cases. Group 2 consisted of 14 luminal type, 1 HER2 positive, 6 TN and 2 TP cases. 15 patients received neoadjuvant therapy (8 in group 1 and 7 in group 2). Follow up data was available for 45 cases (range, 1.4 to 283 mths; median 35 mths). Ipsilateral recurrence was noted in 2 cases in only group 1. 14 cases developed distant metastases (11 vs. 3 in group 1 and 2 respectively; p=0.04). Six patients died of disease (DOD) in group 1 whereas 1 DOD in group 2 (p<0.05). Nine cases tested positive for one of the hereditary breast cancer genes, including 5 in group 1 and 4 in group 2.

| | | Pregnancy – group 1 (n=28) | Postpartum - group 2 (n=23) |
|---|---|---|---|
| **Cases (N)** | | 28 | 23 |
| **Age (yrs)** | Median | 34 | 34.5 |
| | Range | 24 - 43 | 24 - 43 |
| **Primary tumor size (cm)** | Mean | 4.2 | 3.1 |
| | Range | 1.2 – 11.0 | 0.5 – 7.5 |
| **Histologic** | 1 | 0 | 2 (9%) |
| | 2 | 10 (36%) | 5 (23%) |
| **grade** | 3 | 18 (64%) | 16 (68%) |
| **Histologic type** | Ductal | 18 (64%) | 18 (78%) |
| | Lobular | 2 (8%) | 0 |
| | Others[1] | 8 (28%) | 5 (22%) |
| **LVI present (n)** | | 10 (36%) | 9 (39%) |
| **DCIS associated (n)** | | 21 (75%) | 15 (65%) |
| **Multifocal disease (n)** | | 6 (21%) | 5 (22%) |
| **T staging** | T1 | 5 (18%) | 6 (26%) |
| | T2 | 13 (46%) | 13 (57%) |
| | T3 | 3 (11%) | 2 (9%) |
| | T4 | 4 (14%) | 0 |
| | Unknown | 3 (11%) | 2 (9%) |
| **N staging** | N0 | 8 (29%) | 12 (52%) |
| | N1 | 9 (32%) | 3 (13%) |
| | N2 | 6 (21%) | 6 (26%) |
| | N3 | 2 (7%) | 0 |
| | Nx | 3 (11%) | 2 (9%) |
| **M staging** | M0/Mx | 24 (86%) | 23 (100%) |
| | M1 | 4 (14%) | 0 |
| **ER status** | Positive | 15 (54%) | 14 (61%) |
| | Negative | 13 (46%) | 9 (39%) |
| **PR status** | Positive | 16 (57%) | 13 (56%) |
| | Negative | 12 (43%) | 10 (44%) |
| **HER2 status** | Positive | 9 (32%) | 3 (13%) |
| | Negative | 19 (68%) | 20 (87%) |
| **Molecular subtypes** | Luminal | 14 (50%) | 14 (61%) |
| | Triple positive | 4 (14%) | 2 (9%) |
| | HER2 positive | 4 (14%) | 1 (4%) |
| | Triple negative | 6 (22%) | 6 (26%) |

**Conclusions:** No significant difference was observed between group 1 and group 2 cases in relation to age, tumor size, subtype, grade, nodal status, lymphatic invasion, and molecular subtype. The rate for distant metastases and number of cancer related deaths were significantly higher in patients diagnosed during pregnancy ($p<0.05$). However, the cox proportional hazard ratio model did not show any significant difference in survival between the two groups. Further studies to explore the biologic differences in the pregnancy and postpartum breast cancer patients are needed.

## 249 Impact of the 2018 HER2 Testing Focused Update on Classification of dual-probe ISH Groups

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**Disclosures:** Daniel Schmolze: None

**Background:** The 2018 joint ASCO/CAP focused HER2 testing update changed the recommended workflow for interpreting uncommon dual-probe ISH scenarios. These include cases previously deemed positive (by 2013 ASCO/CAP guidelines) because of an elevated HER2/Chr17 ratio or elevated HER2 copy number (groups 2 and 3), as well as cases previously deemed equivocal by HER2 copy number (group 4). The focused update recommends incorporating HER2 immunohistochemical (IHC) results to disambiguate such cases. We performed a retrospective study analyzing paired IHC and FISH results over six years to determine the impact of the 2018 focused update on the classification of ISH group 2-4 cases.

**Design:** We retrospectively identified all breast specimens from our institution for which both HER2 IHC and FISH results were available from January 2013 through July 2018. Based on HER2 copy number and HER2/Chr17 ratio, each FISH result was assigned an ISH group (1-5). IHC score (0, 1+, 2+, 3+) was also recorded. Cases were then interpreted according to ASCO/CAP 2013 guidelines and according to ASCO/CAP 2018 guidelines, with attention paid to cases whose ultimate classification changed.

**Results:** During the study period, 1794 paired IHC/FISH specimens were identified. 28 cases (1.6%) were group 2, 37 cases (2%) were group 3, and 230 (12.8%) were group 4. Based on concurrent IHC findings, cases were reclassified using 2018 criteria as follows: 15 (0.8%) positive to negative, 96 (5.4%) equivocal to negative, 48 (2.7%) positive to “additional work-up required” ,3 (0.2%) equivocal to positive, 131 (7.3%) equivocal to “additional work-up required”. Two remaining positive cases were unchanged.

**Conclusions:** Within groups 2-4, the reclassification rate using 2018 criteria was 16% (293/1794). Only 3 cases (0.2%) were reclassified as positive, 111 (6.2%) were reclassified as negative, and 179 (10%) were reclassified to “additional work-up required”. The largest effect of applying the 2018 criteria to our dataset was a significant reduction in equivocal cases, due to reclassification either as negative (96 cases, 5.4%) or “additional work-up required” (131 cases, 7.3%). We expect a majority of the latter cases to ultimately be classified as HER2 negative following the 2018 recommended procedure of scoring an additional 20 cells, but firm conclusions must await further experience with the 2018 guidelines.

## 250 Molecular Analysis of Triple-Negative Breast Cancers Reveals Diverse Mutational Events in Chromatin Regulation Genes

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**Disclosures:** Christopher Schwartz: None; Matija Snuderl: None; George Jour: None; Farbod Darvishian: None

**Background:** Triple-negative breast cancers (TNBCs) are a heterogeneous subset of breast cancer with a variety of histologic subtypes. An increasing number of evidence links defects in chromatin remodeling machinery to cancer development. Our aim is to analyze the gene expression signatures in a variety of TNBCs with emphasis on genes involved in chromatin regulation.

**Design:** 18 cases of TNBC were selected as follows: 3 apocrine (AP), 3 metaplastic (MP) and 12 no special histologic type (NST). All tumors were grade 3. The median size was 2.5 cm (range 1-4.5 cm). The median age was 59.6 (range 35-82 years). A section of the tumor was compared with corresponding matched normal tissue. Macrodissection for tumor enrichment was performed. DNA was subjected to deep sequencing (average 500x) using our customized panel targeting all exonic and certain select intronic areas in 580 cancer related genes (NGS580) then analyzed using our own bioinformatics pipeline including MuTect2 and Low Freq for single-nucleotide and small indel somatic variants (SNV; indels). Control-FREEC was used for detection of copy number alterations (CNA).

**Results:** Shared pathogenetic mutational events in *TP53* [AP (2/3), NST (10/12) and MP (3/3)] were the most common across all TNBC subtypes. SNV in helicase and kinase domain of *PIK3CA* was identified in 20% of the cases. CNA in known oncogenes and tumor suppressor genes were not identified except *EGFR1* amplification in a single AP case. Tumor mutational burden (TMB) was generally low amongst subtypes (AP, MP, NST: 0.10-0.18, 0.08-0.25, 0.06-0.18 MUT/MB, respectively). The majority of TNBC-NST cases (11/12 cases) were enriched for deleterious SNV events affecting both chromatin remodeling genes including *SMARCA4* (2/12 cases), *ARID1A* (1/12

cases), *DAXX* (1/12 cases), *ITGB3* (1/12 cases), *HIST1H1C* (1/12 cases) and chromatin organization genes including *TET2* (2/12 cases), *TET3* (1/12 cases), *BAP1* (1/12 cases) and *BCOR* (1/12 cases).These events were absent in AP and MP subtypes.

**Conclusions:** TNBC is a genomically heterogeneous group of tumors with low TMB. We identify deleterious events in different genes that are involved in either chromatin remodeling or organization in NST type but not in AP and MP types. These negative findings along with lack of CNA suggest a possible epigenetic mechanism involved in tumorigenesis in AP and MP. Further methylation studies are warranted.

## 251 Breast Cancers with Micropapillary/Tubulopapillary Morphology Demonstrate Frequent DNAH9 Mutations

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**Background:** Dynein Axonemal Heavy Chain 9 (DNAH9) is the heavy chain subunit of axonemal dynein and has been previously implicated in ciliogenesis and cell polarity. Recent studies have shown recurrent mutations of *DNAH9* gene in invasive micropapillary carcinomas by whole exome sequencing. We hypothesized a correlation between the *DNAH9* gene mutation and papillary/micropapillary morphology and sought to analyze the frequency of *DNAH9* mutations in a cohort of invasive breast cancers with micropapillary and tubulopapillary morphology.

**Design:** Four cases of micropapillary carcinoma (MP) and four cases of invasive ductal carcinoma with tubulopapillary features (TP) were selected. Macrodissection for tumor enrichment was performed. DNA was subjected to deep sequencing (average 500x) using our customized panel targeting all exonic and certain select intronic areas in 580 cancer related genes (NGS580) then analyzed using our own bioinformatics pipeline including MuTect2 and Low Freq for single-nucleotide and small indel somatic variants. Control-FREEC was used for detection of copy number alterations.

**Results:** Tumor mutational burden (TMB) was higher in TP (0.2-0.85 MUT/MB) when compared with MP cases (0.05-0.12 MUT/MB). *PIK3CA* events were seen in one of four MPs (25%) and 2 of 4 TPs (50%). Furthermore, both groups demonstrated mutational events in genes involved in cell polarity including deleterious events in *DNAH9* gene in three of four TPs (75%) in the form of splicing and frameshift insertions and in one of four MPs (25%) in the form of single nucleotide variant. Mutations in *DNAH9* gene was the most common shared event. Mutations in *GATA3* (50%) was the most common event in MP cases in the form of frameshift insertion and deletions. TP cases demonstrated mutational events in other cell polarity genes including *CDH5* (25%), *CDH7* (25%), *MYH9* (25%) and *RICTOR* (25%).

**Conclusions:** Recurrent deleterious events in *DNAH9* was noted in 50% (4 of 8) of MP and TP cases. These findings coupled with mutations in other genes involved in cell polarity suggest a correlation between phenotype (micropapillary/tubulopapillary) and genotype in these two subgroups of breast cancers despite their overall distinct mutational landscape.

## 252 Multifocal Breast Cancer: A Clonality Study Using Whole Exome Sequencing

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**Disclosures:** Christopher Schwartz: None; Igor Dolgalev: None; Adriana Heguy: None; Matija Snuderl: None; George Jour: None; Farbod Darvishian: None

**Background:** Multifocal breast cancer (MBC), ductal type, can be hypothesized to arise from a single index tumor, which in turn, gives rise to additional morphologically similar tumors via lymphatic spread analogous to satellitosis/in-transit metastasis in melanoma. Alternatively, MBC can arise from multiple independent foci that may or may not be morphologically similar with each focus carrying its ductal carcinoma in situ. We previously showed that the former MBC (henceforth, MBC1) tend to engender more numerous foci and is significantly correlated with vascular invasion and nodal metastasis compared to the latter MBC (henceforth, MBC2). Herein, we sought to investigate the clonal relationship across separate foci in MBC1 and MBC2 using whole exome sequencing.

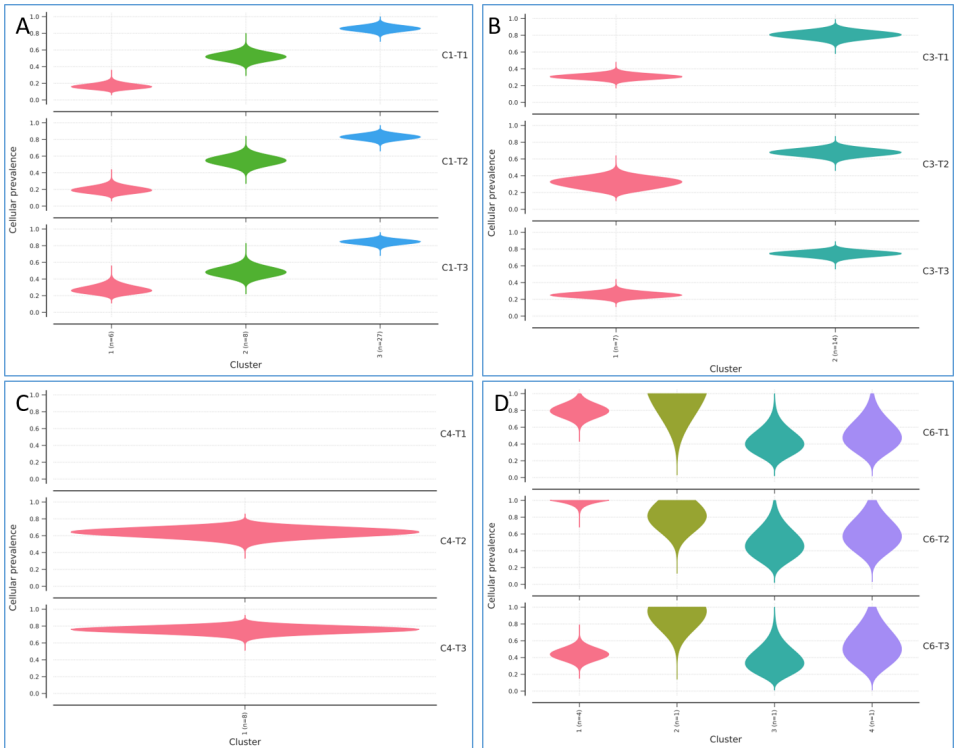
**Design:** We selected three cases of MBC1 (C1 to C3) and 3 cases of MBC2 (C4 to C6). Each case had at least three separate foci of carcinoma. We microdissected the three largest tumors (T1-T3) and one benign lymph node for control from formalin-fixed paraffin embedded (FFPE) tissue blocks (6 sections at 10 micron). Somatic single-nucleotide and small insertion/deletion variants were called with Mutect2. Copy number profiles were calculated using Control-FREEC. Clonal populations were identified and quantified using PyClone.

**Results:** The mean coverage was 33.2 (range=8.2-63.5). C2 was excluded from analysis due to overall low coverage. All 5 cases (C1, C3-C6) showed between 1 to 4 clones/clusters of genes, each harboring between 1 to 27 variants. C1 and C3 showed 3 and 2 clusters,

respectively, shared by all three tumor foci (T1-T3) (figure 1A, 1B). MBC2's C4 and C5 showed only 1 cluster shared by only two tumors with no clone shared by all three foci (figure 1C). C6 showed 4 clusters with 1 variant each shared by all three tumor foci (T1-T3) (figure 1D). The average number of variants in each cluster in C1, C3 and C6 was 13.6, 7 and 1.75, respectively. See table for number of variants.

| Multifocal Breast Cancer Cases with Their Corresponding Shared Gene Clusters | | | | |
|---|---|---|---|---|
| | Cluster 1 Variants (n) | Cluster 2 Variants (n) | Cluster 3 Variants (n) | Cluster 4 Variants (n) |
| Case 1 (T1-T3) | 6 | 8 | 27 | 0 |
| Case 3 (T1-T3) | 7 | 14 | 0 | 0 |
| Case 4 (T2 and T3) | 8 | 0 | 0 | 0 |
| Case 5 (T1 and T3) | 17 | 0 | 0 | 0 |
| Case 6 (T1-T3) | 4 | 1 | 1 | 1 |

Figure 1 - 252




**Conclusions:** As a group, MBC1 (C1 and C3) tend to show a strong clonal relationship across T1-T3 within each case reflected by the size of the clusters/clones. Conversely, MBC2 (C4-C6) shows a rather low clonality. Our findings support the notion that MBC, ductal type, may arise from clonal expansion of one index tumor or independently from multiple foci of carcinoma in situ. Our study also brings the challenges of DNA extraction from FFPE into focus with overall low coverage due to a high fraction of PCR duplicates, which potentially impacts the results.

## 253 Prognostic Significance of CD133 nuclear expression in Invasive Breast Cancer

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**Disclosures:** Young Jin Seo: None; Sun Young Kwon: None; Jung Hyera: None; Yu Na Kang: None; Hye Won Lee: None; Sang Pyo Kim: None; Ji-Young Park: None; Misun Choe: None; Sun Hee Kang: None

**Background:** Cancer stem cells (CSCs) have stem cell-like properties such as tumor initiation, unlimited tumor proliferation, and metastasis. CD133 (pentaspan transmembrane glycoprotein) has been suggested as a useful CSC marker in various tumors. The aim of this study was to investigate the association with CD133 expression and clinicopathological factors in invasive breast cancer (IBC).

**Design:** Immunohistochemical staining of CD133 expression was performed in 292 IBC using tissue microarray. The nuclear and cytoplasmic expression of CD133 were partitively evaluated. We also analyzed the relationship among expression of CD133 and markers of tumor associated macrophages (TAMs) and variable clinical factors. Additionally, overall survival (OS) and disease free survival (DFS) were analyzed.

**Results:** CD133 nuclear expression was found in 28.77 % of IBC. However, CD133 cytoplasmic expression was not associated a significant clinical relevance. CD133 nuclear expression was associated with lower histologic grade ($p$=0.008), estrogen and progesterone receptor positivity ($p$=0.001, 0.007, respectively), and decreased Ki-67 ($p$=0.001). Kaplan–Meier survival analysis revealed CD133 nuclear expression was associated with increased OS ($p$=0.023) and DFS ($p$=0.029). Furthermore, CD133 nuclear expression was conversely associated with 3 markers of TAMs such as CD68 ($p$=0.03), CD11c ($p$=0.01), and CD163 ($p$=0.045) known as tumor progression.

Table 1. Relationship of clinical parameters with cytoplasmic and nuclear CD133 in breast cancer

| Clinicopathologic features | N=292 | CD133 | | | |
|---|---|---|---|---|---|
| | | Nuclear | | Cytoplasm | |
| | | Mean (±SD) | *p*-value* | Mean (±SD) | *p*-value* |
| Age | | | | | |
| <50 | 141 | 2.30 (±2.05) | | 6.22 (±2.95) | |
| ≥50 | 151 | 2.56 (±2.44) | 0.331 | 6.25 (±2.65) | 0.939 |
| Histologic grade | | | | | |
| Grade 1-2 | 101 | 2.92 (±2.30) | | 5.95 (±2.81) | |
| Grade 3 | 191 | 2.18 (±2.21) | 0.008 | 6.38 (±2.79) | 0.210 |
| Tumor size | | | | | |
| ≤20 mm | 113 | 2.68 (±2.08) | | 6.67 (±2.69) | |
| >20 mm | 179 | 2.28 (±2.36) | 0.145 | 5.96 (±2.84) | 0.033 |
| Lymph node metastasis | | | | | |
| Absent | 149 | 2.46 (±2.13) | | 6.25 (±2.72) | |
| Present | 143 | 2.42 (±2.40) | 0.890 | 6.22 (±2.89) | 0.923 |
| Ki-67 | | | | | |
| <14% | 128 | 2.91 (±2.26) | | 6.21 (±2.71) | |
| ≥14% | 164 | 2.07 (±2.20) | 0.001 | 6.25 (±2.87) | 0.906 |
| ER status | | | | | |
| Negative | 94 | 1.81 (±2.30) | | 6.55 (±2.91) | |
| Positive | 198 | 2.74 (±2.19) | 0.001 | 6.08 (±2.74) | 0.178 |
| PR status | | | | | |
| Negative | 80 | 1.86 (±2.23) | | 6.18 (±2.65) | |
| Positive | 212 | 2.66 (±2.24) | 0.007 | 6.25 (±2.86) | 0.829 |
| HER2 status | | | | | |
| Negative | 229 | 2.52 (±2.26) | | 6.24 (±2.84) | |
| Positive | 63 | 2.16 (±2.27) | 0.268 | 6.22 (±2.67) | 0.973 |

*Independent t-test. Abbreviations: SD= standard deviation; ER= estrogen receptor; PR= progesterone receptor; HER2= human epithelial growth factor receptor 2; NA= not available.

Figure 1 - 253

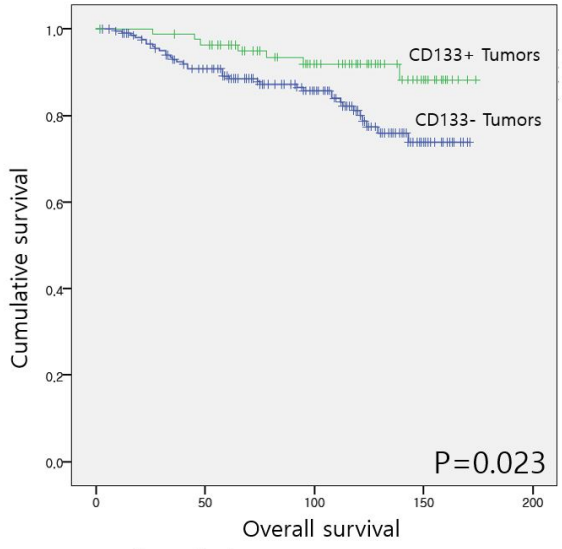


Figure 1. Overall survival: CD133+ vs CD133- Tumor nuclear

Figure 2 - 253

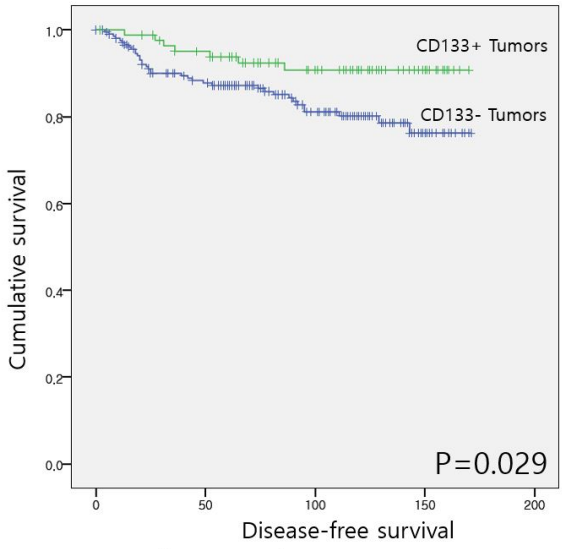


Figure 2. Disease-free survival: CD133+ vs CD133- Tumor nuclear

**Conclusions:** CD133 nuclear expression correlated with favorable prognostic clinical factors and was associated with favorable outcomes of increased OS and DFS in IBC. Our findings therefore revealed CD133 nuclear expression is an independent predictor of good clinical outcomes in IBC. Especially, on the basis of our results, we propose the localization of CD133 expression is important to evaluate tumor prognosis in IBC.

## 254 Genomic Profiling of Basaloid Breast Carcinomas Reveals Recurrent Alterations in CREBBP and Distinct Genetics from Other Triple Negative Breast Cancers

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**Disclosures:** Eliah Shamir: None; Poonam Vohra: None; Gregory Bean: None; Charles Zaloudek: None; Timothy McCalmont: None; Nancy Joseph: None; Rebecca Wolsky: None; Joaquin Garcia: None; Yunn-Yi Chen: None; Gregor Krings: None

**Background:** Triple negative breast cancers (TNBC) comprise a heterogeneous group of tumors. Most are high grade carcinomas of no special type with frequent *TP53* and *PIK3CA* mutations and complex patterns of copy number alterations (CNA). Certain special subtypes are genetically distinct from other TNBC and have indolent behavior. TNBC subtyping thus has clinical significance. Rare TNBC with basaloid features (BCBF) can be distinguished histopathologically but are not readily classifiable into recognized subgroups. We aimed to characterize BCBF by morphology, immunohistochemistry (IHC) and genetic profiling to determine if these tumors comprise a distinct subtype.

**Design:** DNA was extracted from 7 BCBF and matched normal tissue. Next generation sequencing was performed targeting exons of 479 cancer genes and 40 introns. Duplicate reads were removed computationally for allele frequency determination and CNA calling. Single nucleotide variants, insertions/deletions and CNA were evaluated. IHC was performed on 8 BCBF for synaptophysin (SPH), chromogranin (CHR), SOX10, p63, SMA, GATA3, MYB, CD117, Cam5.2, CK7, CK5/6 and Ki67.

**Results:** Mean age was 62 (range 44-89). Mean tumor size was 3 cm; only 1 had nodal metastasis. All tumors had basaloid morphology without dual epithelial-myoepithelial differentiation or other features of adenoid cystic carcinoma. Most (7/8) were grade 2, and 6/8 had carcinoma in situ. None showed necrosis or significant infiltrating lymphocytes. By IHC, all were diffuse SOX10 positive, patchy CK7, Cam5.2 and CK5/6 positive, and negative for p63, MYB, SPH and CHR. SMA was negative in 6/8 and focal in 2/8. CD117 was diffuse in 6/8. GATA3 was negative in 5/7, and weak in 2/7. Mean Ki67 index was 17(±9)%. Recurrent genetic alterations included pathogenic mutations in chromatin modifiers (ChM) *CREBBP*(4/7) and *KMT2D* (2/7), *BCORL1* (2/7), and *NOTCH1* (2/7). Pathogenic mutations in other ChM included *EP300*, *ARID1A,* and *KDM6A* (1 case each). CD274 (PD-L1) was amplified in 1/7. None had *TP53* mutations. No *MYB* or *MYBL1*rearrangements or amplifications were identified. Mean CNA was 7 (range 2-10), with recurrent 6p, proximal 6q, and 17q gain and distal 6q and 14 loss.

| Patient | Age | Tumor size (cm) | SBR grade | CIS | Lymph node | Follow-up (months) | Pathogenic alterations |
|---|---|---|---|---|---|---|---|
| **1** | 44 | 3.8 | 2 | Y | 1/6 | AWD (5) | *CREBBP*, *NOTCH1* (biallelic), *PIK3CA* (subclonal) |
| **2** | 59 | 3.5 | 2 | N | 0/2 | ANED (74) | *CREBBP* (x2), *EP300*, *RHOA* |
| **3** | 56 | 4.7 | 2 | Y | 0/2 | ANED (8) | *CREBBP*, *ARID1A*, *KDM6A* (x2), *BAP1*, *NOTCH1* |
| **4** | 89 | 4 | 2 | Y | 0/1 | ANED (17) | *CREBBP*, *BCORL1*, *KMT2D*, *CD274* amplification |
| **5** | 64 | 2.2 | 2 | Y | 0/1 | ANED (30) | *BCORL1* |
| **6** | 58 | 1.2 | 2 | N | - | ANED (7) | *KMT2D* |
| **7** | 66 | 2.5 | 2 | Y | 0/1 | ANED (2) | *FGFR2*, *TCF7L2*, *MGA* |
| **8** | 59 | 2.1 | 3 | Y | 0/2 | ANED (149) | N/A |

ANED, alive with no evidence of disease; AWD, alive with disease; CIS, carcinoma in situ

**Conclusions:** BCBF have a shared immunoprofile and are morphologically and genetically distinct from other TNBC. Lack of *TP53* mutations and frequent alterations in ChM, especially *CREBBP*, suggest unique biology and may have prognostic and therapeutic implications.

## 255 Comparison of PAX8 Expression in Breast Carcinoma Using MRQ50 and BC12 Monoclonal Antibodies

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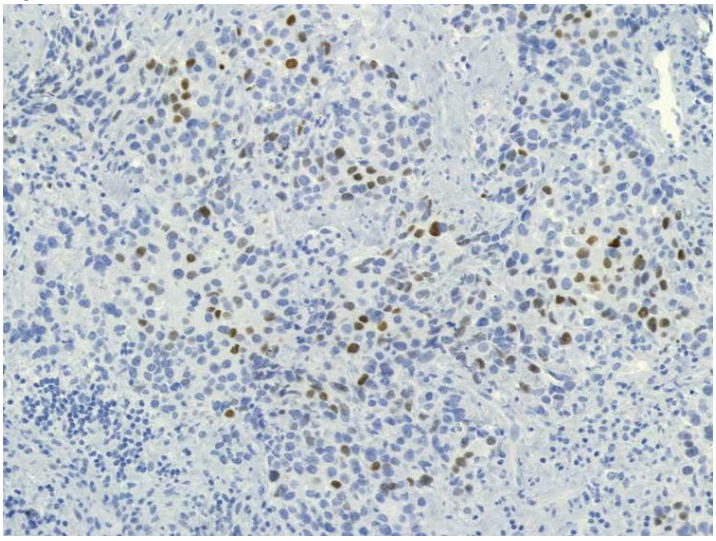
**Disclosures:** Kamaljeet Singh: None; C. James Sung: None; M. Ruhul Quddus: None

**Background:** PAX8 is considered a specific marker for kidney, ovarian and thyroid tissue. Antibody dependent cross-reactivity for PAX8 has been reported in mesothelial, pancreatic and B-cell proliferations. We recently described aberrant PAX8 expression in 2 breast cancer cases. Both tumors were PAX8+ using MRQ50 and PAX8- with BC12 monoclonal antibodies (MAb). There is a limited data on PAX8 expression in breast cancer. In this study we systematically analyze PAX8 expression in breast cancer on full tissue sections using MRQ50 and BC12 PAX8 MAbs.

**Design:** Immunohistochemistry (IHC) was performed on formalin-fixed paraffin-embedded complete tissue sections (4?m thick) from 86 cases of invasive mammary carcinoma. After dewaxing in an oven (60ºCx 1hr) pre-treatment processing was performed in a Dako PT Link module (Tris/EDTA buffer, pH=9). Following peroxidase block (x5 min), slides were incubated with primary antibodies for 10 (MRQ50; Cell Marque) and 20 minutes (BC12; Biocare Medical) respectively. Dako Mouse linker (x15 min) application was followed by Dako EnVision FLEX polymer (x20 min) at room temperature as a secondary antibody. Finally DAB chromogen-substrate system was applied (x10 min). Immunostaining was evaluated at 10x objective and extent (intervals of 10%, 0-100%) and intensity (weak, moderate & strong) of nuclear staining was evaluated in the tumor, benign breast tissue and lymphocytes.

**Results:** With MRQ50 MAb variable PAX8 nuclear positivity was identified in tumors cells in 35/86 (41%) cases. Out of 35 PAX8+ cases, 23 (66%) showed only weak expression in 1-10% cells, 8 (23%) were weakly (5/8) or moderately (3/8) PAX8+ in 11-50% cells and 4 (11%) showed weak PAX8 positivity in > 50% tumor cells. All 3 (3.5%) cases that showed moderate nuclear PAX8 staining with MRQ50 were histologic grade 3. Figure 1 shows example of a moderate PAX8 nuclear immunostaining by MRQ50 MAb. No PAX8 expression was noted in tumor cells or in the lymphocytes with BC12 clone. No PAX8 expression was noted in benign lobules/ducts with either antibody.

Figure 1 - 255



**Conclusions:** Breast carcinomas show aberrant nuclear immunostaining with MRQ50 PAX8 antibody with up to 3.5% cases showing moderately intense expression. The BC12 PAX8 antibody does not cross react with breast carcinoma and lymphocytes. Further studies on PAX8 expression with MRQ50 MAb are needed on metastatic breast carcinomas. During work up of a metastatic carcinoma, weak to moderate PAX8 nuclear expression with MRQ50 clone should be interpreted with caution.

## 256 Adenoid Cystic Carcinoma (ACC) of the Breast: Is a subset of Breast ACC HPV related?

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**Disclosures:** Harmanjot Singh: None; Daniel Lubin: None; Ezra Baraban: None; Ira Bleiweiss: None; Anupma Nayak: None

**Background:** Adenoid cystic carcinoma (ACC), a rare triple negative and basal-like carcinoma of the breast, constitutes <0.1% of all invasive breast carcinomas. However, in contrast to other basal-like triple negative breast carcinomas and salivary gland ACCs, breast ACC in general has a favorable prognosis with 90-100% survival at 10 years. A recently described entity in sinonasal tract known as Human Papillomavirus (HPV)-related multiphenotypic sinonasal carcinoma exhibits overlapping morphologic features with ACC, is associated with high risk HPV strains and unlike other sinonasal carcinomas has favorable prognosis. Here we sought to investigate if there is a possible association between HPV infection and the pathogenesis of ACC in the breast.

**Design:** Cases diagnosed as ACC of breast were retrieved from the pathology archives (1994 – 2018). Ten cases were tested for the presence of HPV using p16 immunohistochemistry (IHC) and an RNA in-situ hybridization (ISH) assay using HPV E6/E7 mRNA probe covering 18 high risk strains of HPV (HPV16,18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82). Appropriate negative and positive controls were evaluated with each case. P16 expression was scored for percentage of positive tumor cells. Both IHC and ISH stains were independently evaluated by 2 pathologists.

**Results:** Of 10 ACC cases, 7 were conventional whereas 3 were solid basaloid variant. HPV-HR18 RNA-ISH assay revealed punctate dot-like brown signals in the nuclei as well as cytoplasm of 2/10 (20%) cases (Figure 1). IHC for p16 demonstrated strong (3+) nuclear and cytoplasmic staining in all 10 cases with percentage scoring as follows: 10% in 2 cases; 40% in 1 case; 50% in 3 cases; 70% in 1 case, and 75% in 3 cases. Of note, 3 cases stained positive for p16 in >75% of tumor cells, the cut-off often used in head & neck cancers. Interestingly, no correlation was observed between positive staining for p16 and HPV RNA-ISH results. Of 3 cases with p16 staining in >75% tumor cells, only 1 case (conventional variant) was positive for HPV-HR signals. Similarly, the second case (solid variant) positive for HPV-HR18 ISH signals showed only 10% tumor cell positivity for p16. Interestingly, the 2 cases positive for HPV were negative for MYB translocation by FISH.

Figure 1 - 256

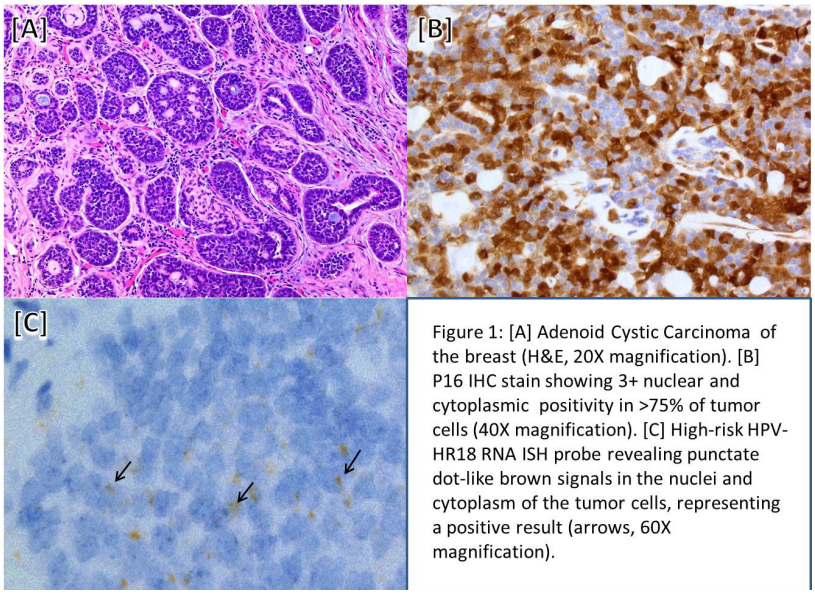


Figure 1: [A] Adenoid Cystic Carcinoma of the breast (H&E, 20X magnification). [B] P16 IHC stain showing 3+ nuclear and cytoplasmic positivity in >75% of tumor cells (40X magnification). [C] High-risk HPV-HR18 RNA ISH probe revealing punctate dot-like brown signals in the nuclei and cytoplasm of the tumor cells, representing a positive result (arrows, 60X magnification).

**Conclusions:** These findings suggest that there may be a potential causative link between high-risk strains of HPV and ACC of the breast. However, further corroboration is required by specific genotyping using PCR method (work in progress).

## 257 Mammary Adenoid Cystic Carcinoma – a Multi-Institutional Canadian Study

Elzbieta Slodkowska[1], Bin Xu[2], Zuzana Kos[3], Anita Bane[4], Maja Barnard[5], Judit Zubovits[6], Pratibha Iyengar[7], Hala Faragalla[8], Phillip Williams[9], Penny Barnes[10], Anna Marie Mulligan[11]
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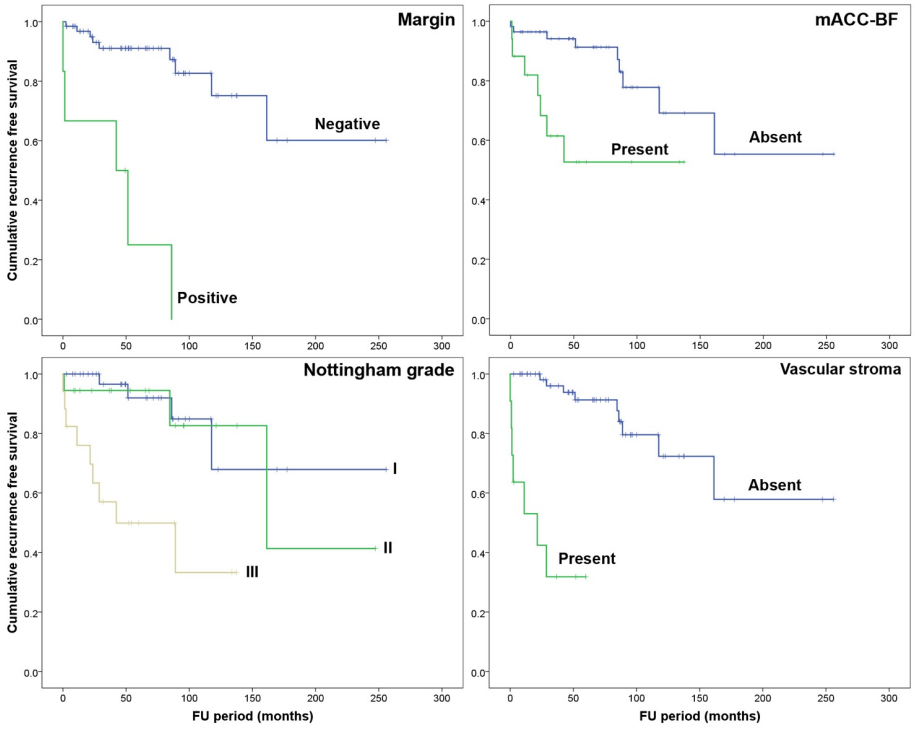
**Disclosures:** Elzbieta Slodkowska: None; Bin Xu: None; Zuzana Kos: None; Anita Bane: None; Maja Barnard: None; Judit Zubovits: None; Pratibha Iyengar: None; Hala Faragalla: None; Phillip Williams: None; Penny Barnes: None; Anna Marie Mulligan: None

**Background:** Mammary adenoid cystic carcinoma (mACC) accounts for 0.1% of breast cancer. Due to its rarity only few studies with a substantial number of cases have been previously reported. Here we present the results of a multi-institutional clinico-pathological study of the largest case series to date.

**Design:** Patients diagnosed with mACC were identified through participating institutions LIS. All available slides were reviewed for the following features: tumor border, growth pattern (including % solid), cellularity, necrosis, Nottingham grade (NG), % of conventional mACC vs. mACC with basaloid features (mACC-BF) as per Rosen (AJSP 26(4): 413–420, 2002), vascular stroma (dense capillary vessels in peri- or intratumoral stroma away from biopsy site), perineural invasion (PNI), lymphovascular invasion (LVI), margin clearance, lymph node (LN) status. Mode of treatment and outcome data were obtained from EPR. The end point was recurrence free survival (RFS) based on combined local recurrence (LR) and distant metastases (DM).

**Results:** 93 mACC were reviewed. Median age at diagnosis was 58 years (range: 32-92), median tumor size was 22 mm (range 5-90). 3 patients were males; 5 had multifocal ACC. FU was available for 73 patients; median 52 months (range 1-349). 6 patients (8%) had LR, 13 (18%) had DM. 2 patients died of disease, one died of other cause, 12 were AWD (7 palliative care) and 58 (79%) NED. 24 (26%) tumors were ≥80% mACC-BF. All patients with involved LN (9, 11%) and all except one with DM had mACC-BF. Factors predictive of RFS on univariate analysis were positive margin, mACC-BF, vascular stroma, NG, PNI, LVI, nuclear grade, solid component >50%, necrosis, LN disease; the first 4 factors remained statistically significant on multivariate analysis (Figure 1). Significant predictors of LR were margin involvement, PNI, LVI and LN disease (on univariate analysis only). Mastectomy, radiotherapy, chemotherapy, multifocality and tumor cellularity were not statistically significant.

Figure 1 - 257




| Multivariate analysis using Cox proportion model | p value | Hazard ratio (HR) | 95% CI of HR |
|---|---|---|---|
| Margin | 0.006 | 767.7 | 6.9-85422.5 |
| mACC with basaloid features (mACC-BF) | 0.037 | 54.2 | 1.3-2313.4 |
| Nottingham grade | 0.019 | 47.4 | 1.9-1192.9 |
| Vascular stroma | 0.005 | 806.3 | 7.6-85936.3 |

**Conclusions:** Margin involvement, NG and vascular stroma strongly predict for RFS and DMFS, and mACC-BF predicts for RFS. PNI is common (27%).

## 258 The Unique Transcriptome of Proliferative Benign Breast Disease

Malvika Solanki[1], Asha Nair[1], Jaime Davila[1], Ethan Heinzen[1], Stacey Winham[1], Derek Radisky[2], Amy Degnim[1], Daniel Visscher[1], Jodi Carter[1]
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**Disclosures:** Malvika Solanki: None; Asha Nair: None; Jaime Davila: None; Ethan Heinzen: None; Stacey Winham: None; Derek Radisky: None; Amy Degnim: None; Daniel Visscher: None; Jodi Carter: None

**Background:** Benign breast disease (BBD) is an independent risk factor for breast cancer (BC). In post-menopausal women, we compared the coding and long non-coding transcriptomes of benign breast tissues with or without proliferative BBD to 1) characterize their RNA expression signatures, 2) gain mechanistic insights into drivers of breast epithelial proliferation in BBD, and 3) identify potential targets to evaluate as biomarkers of early breast carcinogenesis.

**Design:** Cryobanked reduction mammoplasty tissues from age-matched, post-menopausal women (median age: 56 y), were grouped by presence or absence of proliferative BBD into 2 groups: Normal (no BBD, and with complete physiologic lobular involution (LI)) ((N/LI), N=5, and BBD/no LI (BBD/NI), N=5. Following RNA sequencing (Illumina TruSeq Stranded prep/HiSeq 4000), processing (MAP-RSeq v3.0.0 &STAR aligner; hg38), differential expression (DE) analysis (edgeR 2.6.2) identified DE genes from normalized RPKM counts (absolute log2 fold change (FC) > 1; FDR < 0.10). Over-representation analysis [Ingenuity pathway analysis (IPA), Ingenuity® Systems] and gene set enrichment analysis [(GSEA), GeneTrail 2.0] identified significantly-enriched pathways.

**Results:** Between the 2 groups, there were 753 DE genes (coding RNAs: 83% and lncRNA/antisense RNAs: 17%). Among the large group of DE genes, the top BBD/NI-associated up-regulated gene set was enriched in estrogen/bioactive lipid biosynthesis & signaling (e.g. *AREG*, *ESR1*, *ELOVL2*, *CYP4Z1*, *DHRS2, UGT2B11)*. The top N/LI up-regulated DE gene set was enriched in macrophage function and extracellular matrix (e.g. *MARCO*, *PCOLCE2*, *PRG4*). Top BBD/NI canonical pathways included T cell activation and phagosome (P < 0.01). GSEA of the entire gene set (N=15,466; ranked in order of 751 DE genes) identified over 100 KEGG pathways (P < 0.005); the most

significant functionally-grouped as PIK3CA/AKT pathway, adipokine & fatty acid synthesis, mitochondrial energy production, and interleukin signaling. Top DE lncRNAs in BBD/NI included HOTAIR and RMRP ($p < 0.01$).

**Conclusions:** Proliferative BBD has a highly unique transcriptome in post-menopausal breast, enriched in estrogen & bioactive lipid signaling, immune cell activation, lipogenesis & energy production, and functional lncRNAs implicated in BC progression. This pilot study provides novel insights into the metabolic and immune mechanisms associated with epithelial proliferation in BBD, and informs our on-going studies of BBD-related biomarkers of breast carcinogenesis.

## 259 HER2 Positive Breast Cancers with Progression

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**Disclosures:** Malvika Solanki: None; Christopher Chitambar: None; Julie Jorns: None

**Background:** HER2-positive invasive breast cancer was previously associated with poor prognosis. However, HER2-targeted therapies now result in favorable outcomes for most patients. Although uncommon, disease progression following HER2-targeted therapy is particularly problematic and difficult to treat. We sought to evaluate the clinicopathologic features of these tumors.

**Design:** Cancer registry database search (2002-2017) identified HER2-positive breast cancers at our institution. In situ (N=2) and microinvasive carcinomas (N=4) were excluded. All cases of reported HER2-positive primary (T1a or larger), recurrent and metastatic carcinoma (N=167 tumors from 166 patients) were evaluated for clinicopathologic features and outcome. All available slides were reviewed. Tissue microarrays (TMA) were constructed to perform immunohistochemistry (IHC) on cases with sufficient available tumor blocks.

**Results:** Patients were predominantly female (99.4%), with mean age of 53 yrs (23-93). Most (150; 89.9%) were invasive ductal, followed by apocrine (13; 7.8%) and lobular (4; 2.4%). 98 (58.7%) were hormone receptor positive. The method of HER2 positivity was variable. 7 (4.2%) were stage IV at diagnosis, 6 (3.6%) had local recurrence and 14 (8.4%) metastases. Progression and mortality was highest for those with late metastases and stage IV disease at presentation (Table 1). No other feature was significantly associated with disease progression.

73 patients had undergone neoadjuvant chemotherapy (NAC) with anti-HER2 therapy; 35 (47.9%) had no evidence of residual invasive or metastatic carcinoma. Pathologic complete response was most frequently seen in those with positive IHC (IHC positive: 19; 51.3% vs FISH positive: 18; 48.7%) as compared to those without complete response (IHC positive: 8; 22.8% vs FISH positive: 27; 77.2%).

IHC was performed for 86 cases, including 20 with recurrent or metastatic disease (7 with a paired primary tumor). No significant differences were seen between primary and recurrent/metastatic IHC expression. However, 23 (26.7%) were HER2 IHC negative on TMA (Table 2).

Table 1. Comparison of Outcomes of Invasive Breast Cancers (N=168 tumors from 167 patients) Previously Reported to be HER2 Positive

| | Recurrence (N=6) | Metastasis (N=14) | Stage IV at Presentation (N=7) | No Evidence of Disease (N=140) |
|---|---|---|---|---|
| Age (mean (range)) (years) | 51.7 (39-86) | 48 (23-93) | 55.3 (48-60) | 53.9 (29-88) |
| Sex (N, %) | | | | |
| Female | 6 (100) | 14 (100) | 7 (100) | 139 (99.3) |
| Male | 0 (0) | 0 (0) | 0 (0) | 1 (0.7) |
| Laterality (N, %) | | | | |
| Left | 6 (100) | 10 (71.4) | 4 (57.1) | 71 (50.7) |
| Right | 0 (0) | 4 (28.6) | 3 (42.9) | 69 (49.3) |
| Histology (N, %) | | | | |
| Invasive Ductal Carcinoma | 5 (83.3) | 11 (78.6) | 6 (85.7) | 128 (91.4) |
| Invasive Apocrine Carcinoma | 0 (0) | 2 (14.3) | 1 (14.3) | 10 (7.1) |
| Invasive Lobular Carcinoma | 1 (16.7) | 1 (7.1) | 0 (0) | 2 (1.4) |
| Modified Bloom-Richardson Grade (N, %) | | | | |
| 1 | 0 (0) | 0 (0) | 0 (0) | 9 (6.4) |
| 2 | 1 (16.7) | 8 (57.1) | 1 (14.3) | 66 (47.1) |
| 3 | 5 (83.3) | 6 (42.9) | 6 (85.7) | 65 (46.4) |
| Estrogen Receptor (ER) (N, %) | | | | |
| Positive | 4 (66.7) | 10 (71.4) | 4 (57.1) | 75 (53.6) |
| Negative | 2 (33.3) | 4 (28.6) | 3 (42.9) | 65 (46.4) |
| Progesterone Receptor (PR) (N,%) | | | | |
| Positive | 3 (50) | 8 (57.1) | 3 (42.9) | 62 (44.3) |
| Negative | 3 (50) | 6 (42.9) 42.9% | 4 (57.1) | 78 (55.7) |
| HER2 Categorization (N, %) | | | | |
| FISH Amplified; No IHC | 2 (33.3) | 4 (28.6) | 6 (85.7) | 88 (62.9) |
| FISH Amplified; Negative IHC | 0 (0) | 0 (0) | 0 (0) | 1 (0.7) |
| FISH Amplified; Equivocal IHC | 2 (33.3) | 1 (7.1) | 0 (0) | 7 (5) |
| FISH Amplified; Positive IHC | 2 (33.3) | 2 (14.3) | 1 (14.3) | 20 (14.3) |
| Positive IHC; No FISH | 0 (0) | 6 (42.9) | 0 (0) | 24 (17.1) |
| Positive IHC; FISH Non-Amplified | 0 (0) | 1 (7.1) | 0 (0) | 0 (0) |
| Neoadjuvant Chemotherapy (N,%) | | | | |
| Yes | 4 (66.7) | 9 (64.3) | 7 (100) | 62 (44.3) |
| Yes, with HER2-Targeted Therapy | 3 (50) | 6 (42.9) | 7 (100) | 55 (39.3) |
| HER2-Targeted Therapy (N, %) | 5 (83.3) | 10 (71.4) | 7 (100) | 131 (93.6) |
| Pathologic T Stage (N, %) | | | | |
| yT0 | 1 (16.7) | 3 (21.4) | 1 (14.3) | 32 (22.9) |
| yTis | 0 (0) | 1 (7.1) | 1 (14.3) | 6 (4.3) |
| yT1 | 1 (16.7) | 2 (14.3) | 1 (14.3) | 19 (13.6) |
| yT2 | 2 (33.3) | 3 (21.4) | 1 (14.3) | 4 (2.9) |
| yT3 | 0 (0) | 0 (0) | 1 (14.3) | 1 (0.7) |
| T1 | 0 (0) | 2 (14.3) | 0 (0) | 53 (37.9) |
| T2 | 2 (33.3) | 3 (21.4) | 0 (0) | 22 (15.7) |
| T3 | 0 (0) | 0 (0) | 0 (0) | 3 (2.1) |
| Not done | 0 (0) | 0 (0) | 2 (28.6) | 0 (0) |
| Pathologic N Stage (N, %) | | | | |
| yN0 | 3 (50) | 5 (35.7) | 2 (28.6) | 53 (37.9) |
| yN1 | 0 (0) | 4 (28.6) | 2 (28.6) | 8 (5.7) |
| yN2 | 1 (16.7) | 0 (0) | 0 (0) | 3 (2.1) |
| yN3 | 0 (0) | 0 (0) | 1 (14.3) | 0 (0) |
| N0 | 1 (16.7) | 0 (0) | 0 (0) | 49 (35) |
| N1 | 0 (0) | 3 (21.4) | 0 (0) | 18 (12.9) |
| N2 | 1 (16.7) | 2 (14.3) | 0 (0) | 4 (2.9) |
| N3 | 0 (0) | 0 (0) | 0 (0) | 2 (1.4) |
| Not done | 0 (0) | 0 (0) | 2 (28.6) | 3 (2.1) |
| Follow up (mean (range)) (years) (N=165) | 6.0 (4.1-9.0) | 5.3 (1.2-13.0) | 6.3 (1.3-14.7) | 4.9 (0.5-13.1) |
| Progressed (N, %) | 5 (83.3) | 14 (100) | 5 (71.4) | 0 (0) |
| Deceased (N, %) | 1 (0.6) | 4 (28.6) | 3 (42.9) | 0 (0) |

Table 2. Tissue Microarray Immunoprofiles of Selected Primary (M=80) and Recurrent/Metastatic Cancers (N=13) Previously Reported to be HER2 Positive.

| | Primary Tumors (N=80) | Recurrences/Metastases (N=13) |
|---|---|---|
| Cytokeratin 5/6 (N, %) | | |
| Negative (<1%) | 76 (95) | 11 (84.6) |
| Low Positive (=<10%) | 1 (1.3) | 0 (0) |
| Positive (11-49%) | 1 (1.3) | 1 (7.7) |
| Positive (=>50%) | 2 (2.5) | 1 (7.7) |
| Cytokeratin 7 (N, %) | | |
| Negative (<1%) | 3 (3.8) | 1 (7.7) |
| Low Positive (=<10%) | 3 (3.8) | 0 (0) |
| Positive (11-49%) | 2 (2.5) | 0 (0) |
| Positive (=>50%) | 72 (90) | 12 (92.3) |
| GATA3 (N, %) | | |
| Negative (<1%) | 9 (11.3) | 2 (15.4) |
| Low Positive (=<10%) | 4 (5) | 0 (0) |
| Positive (11-49%) | 4 (5) | 3 (23.1) |
| Positive (=>50%) | 63 (78.8) | 8 (61.5) |
| Androgen Receptor (N, %) | | |
| Negative (<1%) | 30 (37.5) | 8 (61.5) |
| Low Positive (=<10%) | 9 (11.2) | 0 (0) |
| Positive (11-49%) | 22 (27.5) | 2 (15.4) |
| Positive (=>50%) | 19 (23.8) | 3 (23.1) |
| HER2 (N, %) | | |
| Negative (0-1+) | 21 (26.3) | 4 (30.8) |
| Equivocal (2+) | 10 (12.5 | 2 (15.4) |
| Positive (3+) | 49 (61.3) | 7 (53.8) |
| HER3 (N, %) | | |
| Negative (0-1+) | 76 (95) | 12 (92.3) |
| Equivocal (2+) | 2 (2.5) | 1 (7.7) |
| Positive (3+) | 2 (2.5) | 0 (0) |
| PDL1 (N, %) | | |
| Negative (<1%) | 68 (85) | 11 (84.6) |
| Positive (=>1%) | 11 (13.8) | 2 (15.4) |
| Positive (=>50%) | 1 (1.2) | 0 (0) |
| Microsatellite Instability (MLH1, MSH2, MSH6, PMS2) Retained (N, %) | 80 (100) | 13 (100) |

**Conclusions:** Features predictive of HER2 positive breast cancer progression remain elusive. However, as previously reported, HER2 protein expression appears most predictive of response to NAC with HER2-targeted therapies, and thus may also be predictive of disease progression.

## 260 Molecular and Clinico-Pathologic Profile of Mucinous Carcinoma with a Micropapillary Pattern (MC-MP)

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**Disclosures:** Thing Rinda Soong: None; Deborah Dillon: None; Tad Wieczorek: None; Stuart Schnitt: None; Beth Harrison: None

**Background:** Mucinous carcinoma with a micropapillary pattern (MC-MP) is a variant associated with more aggressive pathologic features than conventional mucinous carcinoma (MC). It is unclear whether the molecular biology of MC-MP is similar to that of MC and/or micropapillary carcinoma (MPC). We aimed to characterize the genomic and clinico-pathologic profile of MC-MP.

**Design:** Pure mucinous carcinoma (>90% mucin) with a micropapillary pattern (>30% of tumor) diagnosed at our institution between 2002-2018 were included (n=16). A hybrid-capture next generation sequencing (NGS) assay that interrogates the full coding sequences of 447 genes was used to examine tumors with sufficient tissue for mutation (n=14) and copy number variation (CNV) (n=16) analyses.

**Results:** Median patient age was 57 yrs (range: 23-81 yrs). 9 cases presented with a radiologic mass (7 palpable), of which 5 were >2 cm. Most (11) were moderately differentiated and 5 had high nuclear grade. Lymphovascular invasion was present in 6 cases (2 extensive), 4 of which had carcinoma spread to lymph nodes (<3 nodes; 2 macro- and 2 micrometastases). All tumors were ER+/PR+ with 2 being HER2+. With a median follow-up of 4 yrs (range: 0.3-11 yrs), all patients remained alive with no recurrences. The most common pathogenic mutations and gene-level amplifications were alterations involving genes commonly seen in luminal (*GATA3*(5), *FOXA1*(4), *ZNF217*(3)) and mucinous (*SF3B1*(2)) cancers, as well as those more frequently noted in luminal B-like tumors (*CCND1*(4), *MYC*(3), *ERBB2*(2), *TP53*(2)). Other recurrent alterations of unclear biologic significance included genetic changes in *BRIP1*(4), *BRCA2*(3), *RECQL4*(3), and *DOCK8*(3) (**Figure 1**). The most common CNVs were 1q gain(8), 8q gain(4), 8p loss(3),16q loss(3), 17p loss(3). Three of 4 cases with lymph node involvement were associated with multiple (>=3) chromosome arm-level CNVs. One

grade 3 tumor showed genetic instability with 9 chromosome arm/segmental changes (**Figure 2**).
No *FGFR1* or *PIK3CA* alterations previously reported in advanced MC were detected.

Figure 1 - 260

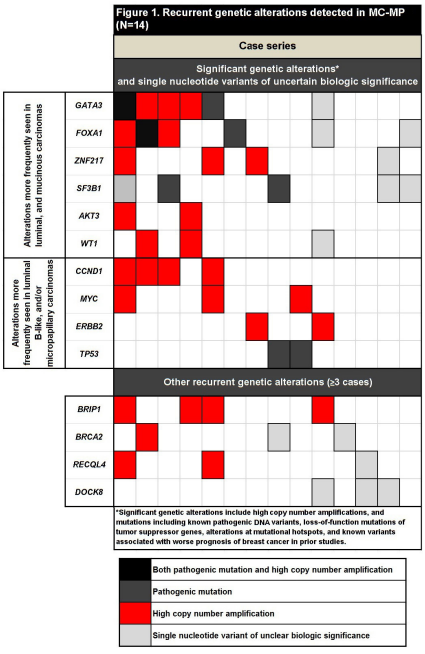
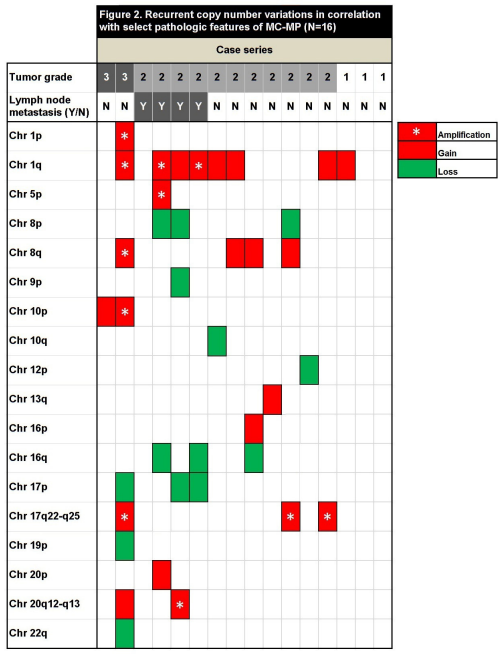



Figure 2 - 260




**Conclusions:** Our study highlights MC-MP as a variant having mixed MPC and MC features in its genomic and clinico-pathologic profile. MC-MP retains an indolent course but exhibits higher rate of lymphovascular invasion and lymph node metastasis than conventional MC. NGS data reveal an overall luminal profile with recurrent alterations known in MC but also enrichment for mutations and CNVs seen in more aggressive forms of luminal breast cancer including MPCs.

## 261 Nottingham Grading of Breast Invasive Carcinoma utilizing Deep Learning Models

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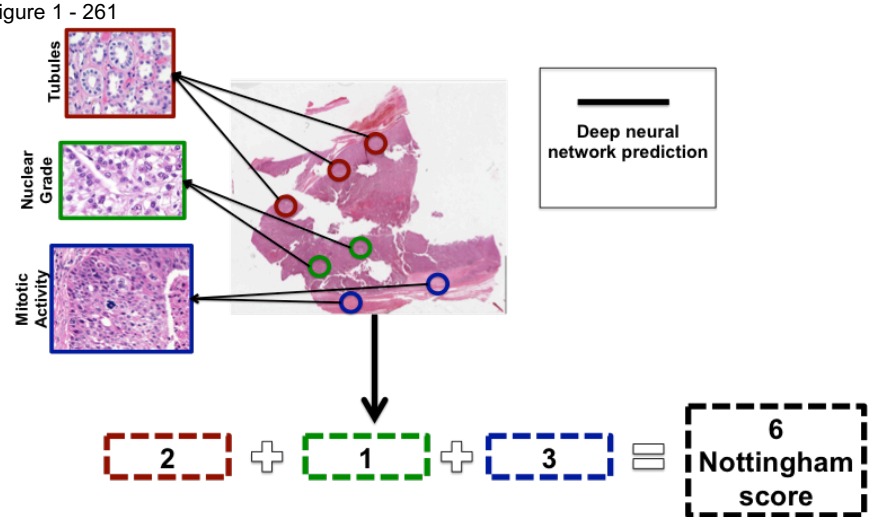
**Disclosures:** Arunima Srivastava: None; Chaitanya Kulkarni: None; Zaibo Li: None; Anil Parwani: None; Raghu Machiraju: None

**Background:** One of the most popular scoring systems for breast cancer is the Nottingham Histologic Score system. This scoring system dictates the assessment of three specific factors, namely tubule formation, nuclear pleomorphism, and mitotic activity. Each factor is scored on a scale of 1-3 and the final score (scale 3-9) is a sum of each factor's individual score. This work aims at building a deep neural network model to individually identify each prognostic factor, evaluate its grade and assign a cumulative Nottingham grade automatically on whole slide image (WSI) of breast invasive carcinoma.

**Design:** The data set for training consists of The Cancer Genome Atlas's digitized histology image compendium for breast invasive carcinoma. It contains over 1000 histology images at 40x magnification, with corresponding patient attributes. In addition, regions annotating areas of high tubular, mitotic and nuclear index are recorded, which allow for training data enrichment. The model architecture utilized for building the subsequent deep learning models follows the traditional "AlexNet" architecture paradigm, which has previously been shown to be successful at modeling histology images. The data set used for validation consists of 94 breast invasive carcinoma cases with 288 WSIs.

**Results:** Our deep learning models identifying and predicting the existence of prognostic factors (high mitotic activity for initial analysis) achieve an accuracy of > 80% and have been validated observationally by subject matter experts. Additionally, deep network models built with mitosis-enriched data, characterize patient outcomes (disease and node stage) effectively. Current results build a foundation for (a) utilizing prognostic factors for disease modeling and (b) using these models to predict distinct staging scores, as they prove to correlate with final diagnostic stages that emanate from these scores.

Figure 1 - 261




**Conclusions:** This work aims at utilizing deep neural networks to automatically predict grading of breast invasive carcinoma. A successful model characterizing the progression and severity of disease is helpful for shortlisting and confirmation of diagnosis and is built as a helpful assist to subject matter experts. Additionally, the framework proposed here utilizes morphological indicators used by pathologists to build a biologically intuitive deep model. This paradigm is intuitively generalizable to other diseases and the corresponding prognostic indicators.

## 262 Retrospective Prognostic Analysis using AJCC 8th Edition Staging System for Triple Negative Breast Cancer

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**Disclosures:** Peng Sun: None

**Background:** AJCC 8th Edition Cancer Staging Manual has updated with a prognostic stage group for breast cancer by incorporating traditional TNM anatomic parameters and biomarkers (ER, PR, Her-2 status, tumor grade, etc.). In this study, we retrospectively compared the prognostic value between the AJCC 8th Editon anatomic and prognostic staging system for triple negative breast cancer(TNBC).

**Design:** We review the data in SYSUCC during 2005-2013 for cases with TNBC. We restaged all cases into anatomic stage (AS) and prognostic stage (PS) groups respectively according to the AJCC 8th Editon staging system. Clinico-pathological features and follow-up data including 5-year disease-free survival (DFS) and overal survival (OS) were collected. The Kaplan-Meier method and log-rank test were applied to evaluate the prognostic significant between different subgroups.

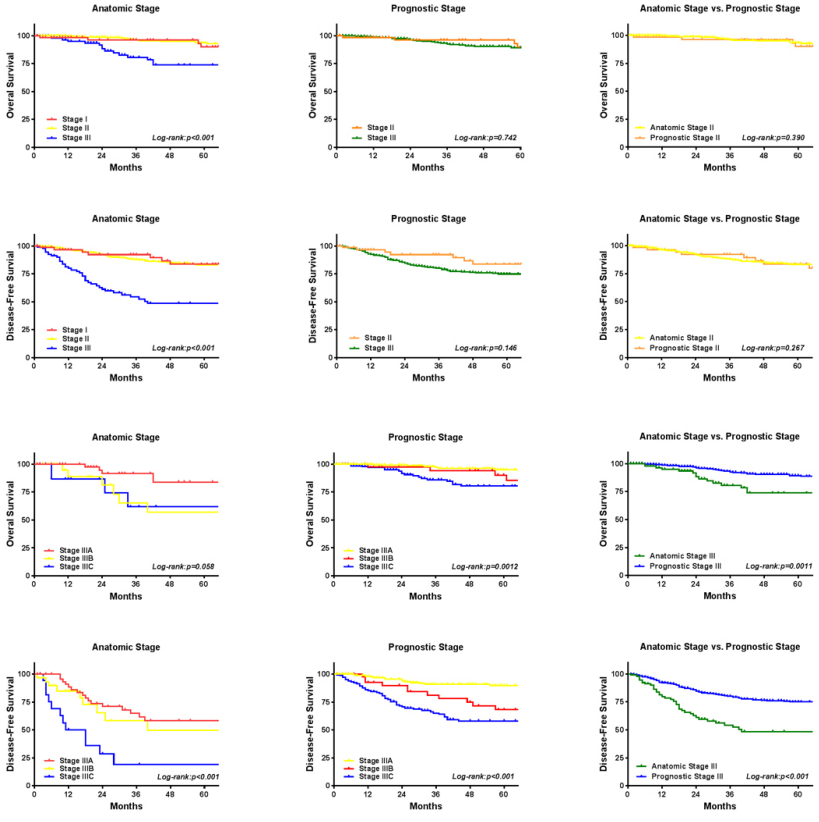
**Results:** The present study enrolled 445 cases of TNBC with 5-year DFS of 76.1% (95% CI 71.4, 80.8) and 5-year OS of 88.8% (95% CI 85.1, 92.5). All AS I tumors were upstaged to PS II groups, and all AS II tumors were upstaged to PS III groups. No case was staged into AS IB, PS I or PS IIB group. AS I, II, and III tumors accounted for 13.9%, 63.6% and 22.5% respectively. PS II and III tumors accounted for 13.9% and 86.1% respectively. Compared to AS groups, a total of 427 (96.0%) cases were upstaged in PS groups, with only 18 (4.0%) cases remained unchanged. Kaplan-Meier curve revealed a better discriminatory ability among all stages in AS groups (5-year DFS, $p<0.001$; 5-year OS, $p<0.001$) than in PS groups (5-year DFS, $p=0.146$; 5-year OS, $p=0.742$). Moreover, 5-year survival increased in PS III groups when comparing with patients in AS III groups on both DFS [80% (95% CI 74.7, 84.4) vs. 59.3% (95% CI 46.2, 70.2), $p<0.001$] and OS [89.9% (95% CI 74.6, 96.2) vs. 73.7% (95% CI 59.6, 83.5), $p=0.0011$]. No prognostic significant was observed between AS II and PS II tumors on either 5-year DFS ($p=0.267$) or 5-year OS ($p=0.390$).

**Table 1. 5-year DFS and OS by Anatomic and Prognosis Stage in TNBC patients**

| | Stage | No. events/No. at risk | 5-year DFS (95% CI) | *p*-value | No. events/No. at risk | 5-year OS (95% CI) | *p*-value |
|---|---|---|---|---|---|---|---|
| **AS** | **I(A)** | **7/62** | **85.4(69.8,93.4)** | **<0.001*** | **4/62** | **89.9(74.6,96.2)** | **<0.001*** |
| | | | | | | | |
| | **II** | **35/283** | **86.5(80.8,90.6)** | **0.001#** | **13/283** | **93.6(88.8,96.3)** | **0.121#** |
| | IIA | 14/181 | 89.8(84.7,94.9) | | 6/181 | 95.1(91.2,99.0) | |
| | IIB | 21/102 | 71.1(60.3,81.9) | | 7/102 | 90.5(83.1,97.9) | |
| | | | | | | | |
| | **III** | **37/100** | **59.3(46.2,70.2)** | **0.015▵** | **15/100** | **73.7(59.6,83.5)** | **0.058▵** |
| | IIIA | 16/50 | 58.3(42.2,74.4) | | 5/50 | 84(72.8,95.2) | |
| | IIIB | 9/32 | 49.9(24.4,75.4) | | 6/32 | 57.0(30.1,83.9) | |
| | IIIC | 12/18 | 19.1(0.3,37.9) | | 4/18 | 61.9(30.3,93.5) | |
| | | | | | | | |
| **PS** | **II(A)** | **7/62** | **85.4(69.8,93.4)** | **0.146*** | **4/62** | **89.9(74.6,96.2)** | **0.742*** |
| | **III** | **72/383** | **80.0(74.7,84.4)** | **<0.001▵** | **28/383** | **89.3(84.6,92.6)** | **0.0012▵** |
| | IIIA | 14/181 | 92.4(88.1,96.7) | | 6/181 | 95.1(91.2,99.0) | |
| | IIIB | 11/48 | 68.2(52.3,84.1) | | 4/48 | 85.6(72.3,98.9) | |
| | IIIC | 47/154 | 58.0(48.4,67.6) | | 18/154 | 80.4(72.0,88.8) | |

* Log-rank test comparing proportions among all stage; # Log-rank test comparing proportions among Stage II;▵ Log-rank test comparing proportions among Stage III. AS, Anatomic Stage; PS, Prognosis Stage; DFS, disease-free survival; OS, overal survival; CI, confidence interval.
No case was classified as AS IB and PS IIB in this study.

Figure 1 - 262




**Conclusions:** The current study showed a poor discriminatory power for the prognostic staging system on prognostic prediction compared to the anatomical staging system in patients with TNBCs.

## 263 Pathologic Discordance of African American and West African Breast Cancer Populations

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**Disclosures:** Hongxia Sun: None; Barbara Nassif: None; Merih Guray: None; Min Jin Ha: None; Kelly Hunt: None; Mamadou Kéita: None; Bakarou Kamaté: None; Khandan Keyomarsi: None; Aysegul Sahin: None

**Background:** The biology of the tumor and hereditary factors contribute to the disparities in breast cancer (BC) outcomes. Triple-negative BC (TNBC), which has more aggressive behavior, is more common among African-ancestry populations, such as African Americans (AA) and West Africans (WA), compared with White/Caucasian Americans population. TNBC is reported to most likely have tumors with predominant tumor infiltrating lymphocytes (TILs). TILs play an important role in predicting the outcome of BC. The purposes of this study are to investigate hormonal receptors subtypes and TILs distribution in BC patients of WA, AA, and non-AA populations.

**Design:** Breast cancer tumor tissues from WA (Mali) were obtained from the Institute of Pathology, University Hospital Point G, Bamako, Mali. H&E stained slides from WA invasive BC were reviewed for histological grading and TILs scoring. TILs scores were given according to the International TILs Working Group 2014 recommendations and recorded as follows: 0-10% score 0; 11-40% score 1; >40% score 2. Immunohistochemical (IHC) stains for estrogen receptor (ER, *Novacastra*), progesterone receptor (PR, *DAKO*), and HER2 (*Ventana PATHWAY*) were performed on formalin-fixed, paraffin-embedded samples from these tumors. The IHC results were scored according to the CAP BC biomarkers guidelines. The results from this WA population were compared with those from the AA and non-AA populations in our institution's database. Fisher's exact tests and t-tests were performed for the comparison of the populations.

**Results:** A total of 122 WA, 36 AA, and 154 non-AA invasive BC were compared. The three populations are matched in patient age (mean age 49 years) and tumor histology grade. ER-negative and TNBC were significantly more common among WA compared with AA and non-AA ($p<0.001$), while TILs scores are lower in WA population ($p<0.001$). Although TILs score was higher in TNBC patients compared with ER positive patients in all the three populations, it was much lower in WA compared with AA ($p<0.05$) and non-AA patients ($p<0.001$) (Table 1).

Table 1: Comparison of pathologic features and TILs among WA, AA and non-AA populations

| | | WA (n=122) | AA (n=36) | Non-AA (n=154) | P value WA vs. AA | P value WA vs. non-AA |
|---|---|---|---|---|---|---|
| ER | | | | | | |
| | Positive (%) | 28 (23.5) | 23 (63.9) | 108 (70.1) | <0.001 | <0.001 |
| | Negative (%) | 91 (76.5) | 13 (36.1) | 46 (29.9) | | |
| PR | | | | | | |
| | Positive (%) | 15 (13) | 17 (47.2) | 78 (50.6) | <0.001 | <0.001 |
| | Negative (%) | 100 (87) | 19 (52.8) | 76 (49.4) | | |
| HER2 | | | | | | |
| | Positive (%) | 8 (6.8) | 8 (22.2) | 30 (19.5) | 0.011 | <0.001 |
| | Negative (%) | 101 (85.6) | 28 (77.8) | 124 (80.5) | | |
| | Equivocal (%) | 9 (7.6) | 0 (0) | 0 (0) | | |
| Histology grade | | | | | | |
| | Low grade (%) | 2 (1.7) | 3 (8.3) | 5 (3.3) | 0.082 | 0.472 |
| | High grade (%) | 117 (98.3) | 33 (91.7) | 147 (96.7) | | |
| Hormone receptor subtypes | | | | | | |
| | ER/PR positive (%) | 32 (26.2) | 17 (47.2) | 95 (61.7) | 0.002 | <0.001 |
| | HER2 positive (%) | 8 (6.6) | 8 (22.2) | 30 (19.5) | | |
| | Triple negative (%) | 66 (54.1) | 11 (30.5) | 29 (18.8) | | |
| TILs score | | | | | | |
| | 0 (%) | 89 (77.4) | 16 (45.7) | 58 (38.9) | <0.001 | <0.001 |
| | 1 (%) | 19 (16.5) | 11 (31.4) | 47 (31.5) | | |
| | 2 (%) | 7 (6.1) | 8 (22.9) | 44 (29.6) | | |
| TILs score in BC subtypes | | | | | | |
| ER/PR positive | 0 (%) | 27 (87.1) | 13 (81.3) | 45 (48.4) | 1 | <0.001 |
| | 1 (%) | 3 (9.7) | 2 (12.5) | 28 (30.1) | | |
| | 2 (%) | 1 (3.2) | 1 (6.2) | 20 (21.5) | | |
| HER2 positive | 0 (%) | 8 (100) | 0 (0) | 9 (33.3) | <0.001 | 0.005 |
| | 1 (%) | 0 (0) | 5 (62.5) | 9 (33.3) | | |
| | 2 (%) | 0 (0) | 3 (37.5) | 9 (33.3) | | |
| Triple negative | 0 (%) | 40 (66.7) | 3 (27.2) | 4 (13.8) | 0.014 | <0.001 |
| | 1 (%) | 15 (25) | 4 (36.4) | 10 (34.5) | | |
| | 2 (%) | 5 (8.3) | 5 (36.4) | 15 (51.7) | | |

**Conclusions:** This study not only shows that there is a strong association between TNBC and West African ancestry, but also that TILs score is much lower in WA BC patients compared with AA and non-AA populations. To our best knowledge, this is the first report regarding to TILs distribution among BC in different populations. These findings could help understand the disparities in BC outcomes.

## 264 Micropapillary pattern in pure mucinous carcinoma of breast is associated with unfavorable prognosis

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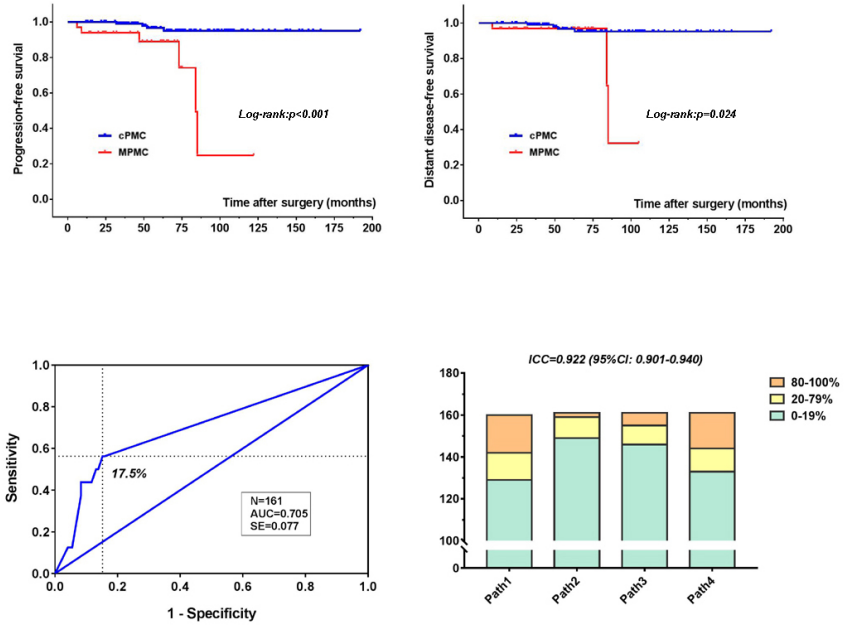
**Disclosures:** Peng Sun: None; Qianyi Lu: None; Rongzhen Luo: None

**Background:** Micropapillary pattern (MP) can be seen in different breast carcinomas and its prognostic significance is uncertain. While invasive micropapillary carcinoma possesses aggressive behavior and poor prognosis, a similar pattern may occur in pure mucinous carcinoma (PMC) named micropapillary variant of mucinous carcinoma (MPMC) and its prognostic significance remains controversial.

**Design:** A retrospective review of 161 cases of PMC diagnosed during 2005-2015 by four pathologists was conducted. Clinico-pathologic features including age, tumor size, nuclear grade, lymphovascular invasion(LVI), lymph node metastasis(LNM), MP percentage(MP%), AJCC TNM stage and ER, PR, HER-2, Ki67 expression were evaluated. Intraclass correlation coefficient(ICC) was used to estimate the agreement among four pathologists for MP%. The patients were followed up for 12-192 months (median 63 months). 21-gene Recurrence Score Assay was applied in selected conventional PMC(cPMC, n=13) and MPMC(n=14) cases that were pT

**Results:** MPMC was identified in 32(19.9 %) of the 161 PMC. Compared to cPMC, MPMC occurred in younger age (median age, 42 vs. 46 years; p=0.003), exhibited higher nuclear grade (grade 1, 3.1 vs. 75.2%; grade 2, 71.9 vs. 24%; grade 3, 25 vs. 0.8%; p<0.001), more frequent LVI(50% vs. 9.3%; p<0.001), LNM(46.9% vs. 23.2%; p<0.001), higher HER2 overexpression(25.0% vs. 2.3 %; p<0.001). Higher risk of local recurrence (9.4 vs. 0%; p<0.001) was observed in MPMC. There was no difference in tumor size, ER, PR expression, distant metastasis and death of tumor when compared to cPMC. Kaplan–Meier analysis showed that MPMC patients had a decreased progression-free survival (PFS)(p<0.001) and distant disease-free survival(DDFS)(p=0.024) than cPMC patients. In univariate analysis, MPMC morphology was a prognostic indicator for PFS(HR=12.0, p<0.001) and DDFS(HR=5.0, p=0.041), but not OS. Nuclear grade was the only independent factor for PFS or DDFS in multivariate analysis. The optimal cut-off value of MP% was 17.5%(AUC=0.705; sensitivity, 56.3%; specificity, 84.8%). The ICC for agreement among pathologists was 0.92(95 % CI 0.901–0.940; p<0.001). Compared to cPMC, a higher Recurrence Score was also observed in MPMC patients(41.71±5.54 vs. 23.21±3.29, p=0.009).

Figure 1 - 264




**Conclusions:** MP in breast cancers may contribute to aggressive behavior and indicate unfavorable prognosis in PMC. Moderate to high nuclear grade and the exact MP% should be assessed in the diagnosis of MPMC, which may represent an aggressive subtype of PMC.

## 265 Characteristics of T-Lymphocytes in the Immune Microenvironment of Breast Ductal Carcinoma in situ

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**Disclosures:** Aye Aye Thike: Non; Valerie Koh: None; Nur Diyana Md Nasir: None; Puay Hoon Tan: None

**Background:** Ductal carcinoma *in situ* (DCIS) of the breast is a heterogeneous preinvasive cancer. With mammography being widely available for breast cancer screening, it accounts for 20–25% of newly diagnosed breast cancers. Although women with pure DCIS have excellent prognosis, those with subsequent invasive recurrences have an increased risk of breast cancer-specific death. A deeper understanding of the characteristics of T lymphocytes around DCIS enables insights into reliable immune markers that may predict risk of DCIS recurrence and progression. We aimed to investigate the role of T cell markers: CD8, CD4 and FoxP3 in defining behavior.

**Design:** The cohort comprised 198 DCIS cases including 101 tumors with known recurrent disease and 97 non-recurrent DCIS as controls. Immunohistochemistry was performed on standard sections using antibodies to ER, PR and HER2 to define triple negativity and T cell markers;CD8, CD4 and FoxP3. Positive biomarker expression of CD8 and CD4 was defined as membranous staining of 10% or more of immune infiltrates and FoxP3 as nuclear staining of at least 1% of the immune population. Expression of T cell markers was correlated with pathological parameters and outcome.

**Results:** CD8, CD4 and FoxP3 expression in immune infiltrates surrounding DCIS was observed in 45% 23% and 12% of cases.Thirty six (18%) tumors showed high $CD4^+/CD8^+$T cells ratio (?1). High nuclear grade was significantly associated with CD8, CD4 and FoxP3 expression (p=0.001, p=0.005 and p=0.050). CD8 and FoxP3 expression was associated with presence of microinvasion (p=0.006 and p=0.015). ER negativity was correlated with CD8, CD4 and FoxP3 expression (p=0.001, p=0.002 and p=0.003). CD8 expressed more in T cells surrounding HER2 positive DCIS (p=0.008). On Kaplan-Meier analysis, worse DFS for ipsilateral invasive recurrence was observed in patients with tumors harboring high $CD4^+/CD8^+$T cell ratios (p=0.020). This finding was confirmed by multivariate analysis (95%CI 1.268-7.128, HR 3.007, p=0.012). High FoxP3 expression showed a trend for early development of ipsilateral invasive recurrence (p=0.114).

**Conclusions:** Our study demonstrates that subtypes of T lymphocytes in immune cells around DCIS were associated with poorer prognostic parameters. Higher $CD4^+/CD8^+$T cell ratios independently predicted disease progression. The characteristics of T lymphocytes may serve as potential predictive and prognostic biomarkers for identifying high risk patients for stratified treatment.

## 266 Mucocele-Like Lesion of the Breast Diagnosed on Core Biopsy: Histologic and Clinical Analysis of 73 Cases with Focus on Features Associated with Upgrade

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**Disclosures:** William Towne: None; Paula Ginter: None

**Background:** Breast mucocele-like lesions (MLL) are rare and characterized by mucin filled cysts and stromal mucin extravasation. Recent studies show relatively low rates of upgrade from core biopsy to excision. Given the low incidence of MLL, published data is limited and the optimal management is unclear. Our aim was to evaluate features associated with upgrade rate of MLLs diagnosed on core biopsy.

**Design:** MLLs diagnosed on core biopsy from 1998-2018 were retrieved. MLLs associated with invasive carcinoma or ductal carcinoma in situ carcinoma (DCIS) were excluded. Archived slides and clinical records were reviewed. The following parameters were evaluated: presence of atypia [atypical ductal hyperplasia (ADH) or atypical lobular hyperplasia (ALH)], presence and type of calcifications (coarse vs. fine), and mucocele type [classic (C-MLL), duct ectasia-like (DEL-MLL), or cystic mastopathy-like (CML-MLL)]. Subsequent excisions were reviewed and upgrade rate was evaluated.

**Results:** 73 core biopsy cases of MLLs were identified. MLL were primarily detected by radiologic calcifications (68/73; 93.2%), rarely as a palpable mass (3/73; 4.1%) or MRI abnormality (2/73; 2.7%). 7/73 (9.6%) MLLs were incidental (not the apparent biopsy target). Of the 73 cases, 40 (54.8%) lacked atypia and 33 (45.2%) were associated with atypia (ADH=32, ALH=1). A majority were C-MLL (55/73; 75.3%) with fewer DEL-MLL (15/73; 20.6%) and CML-MLL (3/73; 4.1%). 23/73 (31.5%) showed coarse calcifications only, 9/73 (12.3%) showed fine calcifications only, 34/73 (46.6%) showed both coarse and fine calcifications, and 7/73 (9.6%) showed no calcifications. Subsequent excision was available for review in 17 of 40 MLLs without atypia. 2/17 (11.8%) were upgraded to DCIS and 1/17 (5.9%) showed ADH. Subsequent excision was available for 22 of 33 MLLs with atypia. Of these, 6/22 (27.3%) were upgraded to DCIS, 2/22 (9.1%) showed lobular carcinoma in situ and 8/22 (36.4%) showed atypia (ADH=7, ADH and ALH=1). No cases were upgraded to invasive carcinoma. All eight cases upgraded to DCIS were C-MLL. Five were associated with coarse calcifications only and three with both coarse and fine calcifications.

**Conclusions:** A vast majority of MLLs present as calcifications and nearly 50% are associated with atypia. Upgrade to DCIS is twice as frequent in MLLs with atypia versus those without. Classic-MLL may be associated with a higher upgrade rates than DEL- or CML-MLL variants. Coarse calcifications are more commonly observed in upgraded MLLs than fine.

## 267 Breast Core Biopsy May Determine pT Category and Be the Only Specimen Available for Ancillary Testing

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**Disclosures:** William Towne: None; Jordan Baum: None; Paula Ginter: None

**Background:** The fundamental crux of breast cancer staging is the TNM system in which the pathologic stage is dependent on gross and microscopic evaluation of breast specimens. While in many instances, there is ample residual tumor to determine largest tumor dimension and final T categorization in the surgical resection specimen, advances in imaging modalities have led to improved detection of clinically undetectable tumors (i.e. <2 cm) that may be substantially or entirely removed in the core biopsy specimen. Additionally, ancillary studies, such as molecular testing often require accurate selection of the case/block with the largest tumor volume to avoid failure due to lack of required DNA/RNA. The aim of the study was to determine the frequency that core biopsy determined largest tumor size and how often it affected pT categorization.

**Design:** Consecutive primary invasive breast carcinomas were identified in the pathology archive over a 2.7 year period (2016-2018) at New York-Presbyterian Hospital/Weill Cornell Medicine. Cases treated with neoadjuvant therapy were excluded. At our institution, measurement of contiguous tumor on core biopsy specimens is routinely reported. Pathology and clinical records were reviewed and tumor size in core biopsy and excision were collated.

**Results:** The final cohort consisted of 840 invasive carcinomas (T1mi: 51, T1a: 146, T1b: 251, T1c: 271, T2: 109, T3: 8, and T4: 4). In this cohort, the largest tumor size was documented on the core biopsy in 87 cases (10.4%). In 62 cases (7.4%), the assigned pT category reflected the tumor size in the core biopsy specimen. Amongst the cases where the pT category was determined on core biopsy (**see Table**), a majority showed no residual invasive carcinoma on excision (35/62, 56%). Additionally, for those cases determined on core, pT1a tumors were the most frequently downgraded (35/62, 56.5%), T1b tumors were almost exclusively downgraded to T1a (15/16, 93.6%) and T1c tumors were most frequently downgraded to T1b (4/5, 80%). As expected, no T2 or T3 tumors downgraded on excision.

| | | Accurate pT Category Determined on Core Biopsy (n=62) | | | |
|---|---|---|---|---|---|
| | | **T1mi** | **T1a** | **T1b** | **T1c** |
| **Inaccurate pT Category Based on Resection Specimen** | **T0/Tis** | 6 (9.7%) | 28 (45.2%) | 1 (1.6%) | |
| | **T1mi** | | 7 (11.3%) | | |
| | **T1a** | | | 15 (24.2%) | 1 (1.6%) |
| | **T1b** | | | | 4 (6.4%) |

**Conclusions:** Given the importance of appropriate assignment of pT category in clinical management decisions and the influence of tumor size on case/block selection for molecular testing, routine reporting of tumor size in core biopsies is of clinical value and ought to be encouraged.

## 268 Breast Papillary Carcinoma Diagnosed on Core Biopsy has High Upgrade Rate to Invasive Carcinoma on Excision

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**Disclosures:** Kevin Van Smaalen: None; Kathleen Len: None; Xiaoxian Li: None

**Background:** Papillary carcinoma is an uncommon diagnosis in breast core biopsies. It is an umbrella terminology of in situ carcinoma including intraductal papillary carcinoma, encapsulated papillary carcinoma, papilloma extensively involved by ductal carcinoma in situ and solid papillary carcinoma. This study examined the upgrade rate to invasive carcinoma and lymph node status in the excisional specimen.

**Design:** We retrieved 183 cases from our archives. After review, we included 41 consecutive breast biopsies with papillary carcinoma without invasion. All 41 lesions were excised at our institution. The other cases either had invasive carcinoma on the core biopsy or did not meet our morphologic criteria.

**Results:** Of the 41 cases, 36/39 (92%) were estrogen receptor positive (ER+) and 33/39 (85%) were progesterone receptor positive (PR+). Of the 41 cases that were excised, 16 (39%) had invasive carcinoma upgrade in the excisional specimen including 2 microinvasive carcinoma (≤1 mm); 21 had lymph node excision including 12 of the 16 invasive carcinoma cases. None had lymph node metastasis.

**Conclusions:** The upgrade rate to invasive carcinoma of papillary carcinoma diagnosed on core biopsies is high. Complete excision is recommended for these patients. The role of sentinel lymph node biopsy is unclear and will require further evaluation since none of the patients had lymph node metastasis.

## 269 Significance of Classifying Breast Papillary Carcinoma Diagnosed on Core Biopsy

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**Disclosures:** Kevin Van Smaalen: None; Kathleen Len: None; Xiaoxian Li: None

**Background:** Papillary carcinoma is an uncommon umbrella terminology we use in breast core biopsy. The differential diagnoses include intraductal papillary carcinoma (papillary DCIS), encapsulated papillary carcinoma, solid papillary carcinoma and papilloma extensively involved by DCIS. The significance of accurately classifying papillary carcinoma on core biopsy is unclear.

**Design:** We retrieved 27 consecutive biopsies with the papillary carcinoma without invasion. Based on morphologic evaluation, we further classified the lesions to solid papillary carcinoma, intraductal papillary carcinoma/encapsulated papillary carcinoma and papilloma extensively involved by DCIS. Upgrade rate to invasive carcinoma and lymph node status in the excision were evaluated.

**Results:** Of the 27 cases, 18 were classified as intraductal papillary carcinoma/encapsulated papillary carcinoma; 6 solid papillary carcinoma and 3 papilloma extensively involved by DCIS. Because of the significant morphologic overlap, intraductal papillary carcinoma and encapsulated papillary carcinoma were classified as one group. Of the 18 intraductal papillary carcinoma/encapsulated papillary carcinoma, 5 (28%) were upgraded to invasive carcinoma on excision; 4 of 6 (67%) solid papillary carcinoma upgraded to invasive carcinoma; and 1 of 3 (33%) papilloma extensively involved by DCIS upgraded to invasive carcinoma. Fourteen patients had lymph node excision and none had lymph node metastasis.

**Conclusions:** The upgrade rate to invasive carcinoma in each group is high. There is no clinical significance to classify papillary carcinoma diagnosed on core biopsy. Breast papillary carcinoma diagnosed on core biopsy should be excised with/without sentinel lymph node biopsy.

## 270 Inter-observer reproducibility of classical lobular neoplasia (B3-lesions) in preoperative breast biopsies. A Study of the Swiss Working Group of Breast- and Gynecopathologists

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*[1]University Hospital, Zurich, Switzerland, [2]University Hospital Zurich, Zurich, Switzerland, [3]Pathology Institute Länggasse Ittigen-Bern, Bern, Switzerland, [4]Institut fur Pathologie Kantonsspital, Munsterlingen, Switzerland, [5]Pathology Institute Regenbogen, Kreuzlingen, Switzerland, [6]Triemli-Spital, Zurich, Switzerland, [7]Pathology Institute Länggasse Ittigen-Bern, Ittigen, Switzerland, [8]Institute of Pathology and Molecular Pathology, University Hospital Zurich, Zurich, Switzerland,[9]University Bern, Bern, Switzerland*

**Disclosures:** Zsuzsanna Varga: None; Chantal Pauli: None; Achim Fleischmann: None; Tilman Rau: None

**Background:** Classical form of lobular neoplasia (LN) spans a spectrum of disease, including atypical lobular hyperplasia (ALH), lobular carcinoma in situ (LCIS), classical lobular neoplasia (LN) and the three tiered classification of lobular intraepithelial neoplasia (LIN 1,2,3). This study aimed to assess inter-observer variability between breast – and gynecopathologists of classical lobular neoplasia (LN) (B3-lesions) of preoperative breast biopsies.

**Design:** A retrospective, observational, cross-sectional study was conducted. A total of 40 preoperative digital images of breast core/vacuum biopsies were analyzed by 8 experienced breast – and gynecopathologists. Evaluation criteria were ALH, LCIS, LN classic, LIN-1, LIN-2, LIN-3 focal B3 (one TDLU), extensive B3 (>one TDLU). Statistical analyses were conducted using the kappa index and chi square test. Digital scanned images were provided to each participant. Agreement between the categories was defined as at least 6 out of 8 pathologists (cut-off of 75%) being concordant on the same category.

**Results:** The highest agreement between 8 pathologists was reached using the category classical lobular neoplasia (LN, classical), 26/40 cases were diagnosed as such. Agreements in other categories was low or poor: 12/40 (ALH), 9/40 (LCIS), 8/40 (LIN-1), 8/40 (focal B3), 4/40 (LIN-2), 2/40 (extensive B3). Chi-Square statistic (classical LN versus the other groups) was significant (p=0.001137).

**Conclusions:** Our data suggest that in Switzerland the most reproducible diagnosis among expert pathologists for B3 lobular lesions is the category of classical LN. These data further support lack of consistent data regarding analysis of retrospective studies using different terminologies. Validation of reproducible nomenclature is warranted in further studies. This information is useful especially in view of retro- and prospective data analysis with different diagnostic categories.

## 271 Tumor Infiltrating Lymphocyte Volume (TILV) Predicts the Disease Free Survival in Invasive Breast Cancer

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*[1]The University of Texas Health Science Center at Houston, Houston, TX, [2]Bellaire, TX, [3]University of Texas Houston, Houston, TX, [4]UT Health Science Center at Houston, Houston, TX, [5]The University of Texas MD Anderson Cancer Center, Houston, TX, [6]The University of Texas at Houston, Houston, TX*

**Disclosures:** Elmira Vaziri Fard: None; Yasir Ali: None; Xiaohong Iris Wang: None; Michael Covinsky: None; Karan Saluja: None; Lei Wang: None; Songlin Zhang: None

**Background:** Evaluation of tumor infiltrating lymphocytes (TILs) has been recommended as one of the prognostic cancer biomarkers in several malignancies, however, the current TILs evaluation does not consider the tumor stromal volume. Previously, we proposed to use the tumor infiltrating lymphocyte volume (TILV) to reflect the true tumor immunity, and found that TILV had better predictive value on both pathologic complete response (pCR) and overall survival in triple negative breast cancer. The goal of current study was to validate the TILV predictive value in breast cancer with mixed breast cancer types.

**Design:** The TILs and TILV were evaluated on 100 breast cancer cases with mixed histologic types including 74 invasive ductal carcinoma, 14 invasive lobular carcinoma, 1 mucinous carcinoma, and one intracystic papillary carcinoma. Pathologic information of tumors was collected by reviewing the pathologic reports, and the follow-up information such as recurrent/metastasis and death was collected by medical chart review. T-test, Chi-Square, and Kaplan-Meier were used for statistical analysis.

**Results:** As expected, high grade (G3) and triple negative breast cancers had higher TILs and TILV than low grade and ER+ breast cancers. TILs did not show significant predictive value for the disease-free survival by neither two tiers (TILs ?60% vs <60%) nor three tiers (TILs ?60%, 20%-<60%, and <20%) analysis. However, high TILV showed significant better survival (p=0.046) than low TILV (two tiers TILV?1300 vs TILV <1300). The patient age, tumor size and lymph node status were not significantly different between the two groups. The TILV?1300 group actually had more ER- and grade G3 cancer than TILV<1300 group. Using 3 tiers analysis (TILV?1600, TILV600-TILV1600, and TILV<600), TILV also showed better survival prediction than using TILs.

Figure 1 - 271

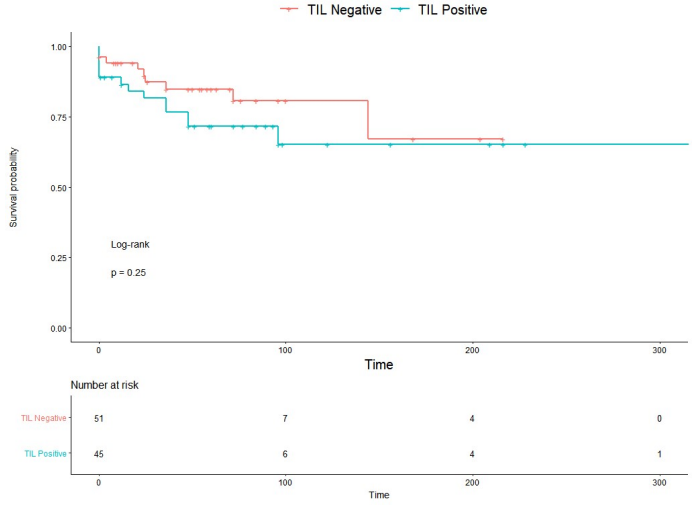
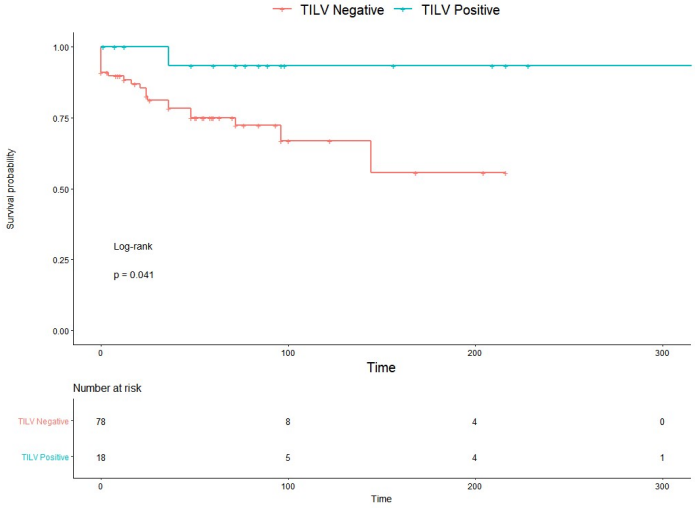



Figure 2 - 271




**Conclusions:** TILV includes both TILs and the stromal volume in the calculation for tumor immunity compared to evaluation of TILs alone, and TILV shows better predict value on the disease free survival of breast cancer. High TILV can even overcome some conventional high risk factors such as negative ER and high tumor grade on predicting the survival. We recommend routine evaluation of TILV on all breast cancer cases.

## 272 Ki67 Scoring In Breast Cancers Using a Proposed Mobile Application Based Scoring Algorithm, It's Impact on Patient Outcomes and Comparison with Automated Digital Image Analysis (DIA) software. Can We Standardize Ki67 Scoring?

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**Disclosures:** Saranya Venkatesh: None; Rosina Ahmed: None; Sanjit Agarwal: None; Namrata Maity: None; Indu Arun: None

**Background:** Ki67 labelling index(LI) is used along with ER, PgR and HER2 immunohistochemistry(IHC) as a surrogate for molecular classification of breast cancer. However, it's utility to distinguish luminal subtypes of breast cancer is limited by lack of standardisation of Ki67 scoring. The aims of this study are to (1) evaluate the reproducibility of Ki67 LI between 2 scorers using global methods proposed by the International Ki67 Working Group with the aid of a phone based manual counting Android application (app), (2) To correlate the reproducibility of app scores with Automated Digital Image Analysis (DIA), (3)To derive an optimal Ki67 cut off value and evaluate its prognostic implication in our cohort.

**Design:** Ki 67 LI of 395 ER positive HER2 negative breast core biopsies diagnosed at our institute between 2012 and 2016 was evaluated. Ki67 scoring on 103 core biopsies was done by 2 scorers by 2 methods (1) Unweighted global score (4 fields of 100 cells each) and (2) Weighted global score (GW) (weighted by estimated percentages of total area) using the app scoring tool. Intra-class correlation coefficient (ICC) was used to assess the absolute agreement between the scorers.The significance of difference between Ki67 scores by app scoring and eye ball estimate and also between app scores and DIA scores (using LEICA APERIO VERSA scanning system) were determined by One-Sample t Test. ROC curve analysis was performed on 395 cases (scored by single observer by global methods) to obtain the optimal Ki67 cut-off. The association of Ki67 and PgR values with other clinico-pathological variables and disease free survival (DFS) was done by Multivariate Cox proportional hazards regression model and Kaplan Meier method respectively.

**Results:** ICC for 103 core biopsies between 2 observers showed an excellent degree of reliability between scorers with both GW and GUW methods (GW ICC = 0.964, *P* <0.001 and GUW ICC = 0.966, *P* <0.001). The mean differenence between app method and DIA was 3.17 while that between app method and eyeballing was 7.76. ROC curve analysis showed that Ki-67 of 26.6% (AUC 0.705; p < 0.001) had the best sensitivity and specificity to predict relapse.The multivariate analysis confirmed that Ki67 and PgR were independently associated with DFS.

Multivariate survival analysis using Cox proportional hazards regression models.

| Variable | Disease free survival (DFS) | | | |
|---|---|---|---|---|
| | Category | Hazard ratio | 95% CI | p value |
| pT stage | T1 vs T2-T4 | 1.192 | 0.481-2.949 | 0.705 |
| P N stage | N0 vs N1-3 | 0.073 | 0.016-.0848 | 0.001 |
| Lymphovascular invasion | Absent vs Present | 1.024 | 0.256-4.098 | 0.974 |
| Histological grade | G1 vs G2-G3 | 0.359 | 0.117-1.101 | 0.073 |
| Histological subtype | IDC vs Others | 1.378 | 0.179-10.629 | 0.758 |
| Age | <50 vs > 50 | 0.949 | 0.909-0.991 | 0.017 |
| Ki67 index<br><br>(App method) | <26% vs >26% | 0.172 | 0.045-0.660 | 0.010 |
| PR category | <20% vs >=20% | 3.652 | 1.078-12.368 | 0.037 |

Figure 1 - 272

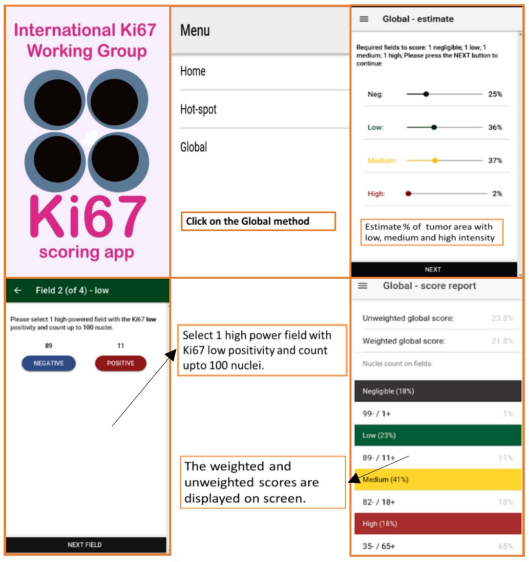
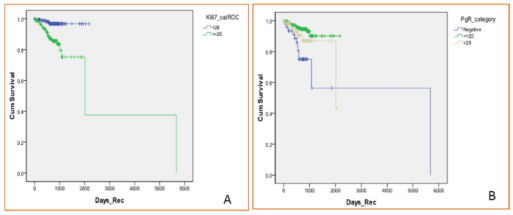
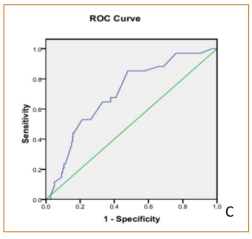



Figure 2 - 272




**A.**Kaplan Meier curve showing DFS varying with the Ki67 below and above the cut-off value. (P value= 0.000)
**B.**Kaplan Meier curve showing survival distributions for the different levels of PgR_category. (<20% vs >=20%)




**C.**Optimal cutoff ki67 is 26.6, specificity 0.499, and sensitivity 0.853. Ki67 Cut-off value obtained based on ROC curve analysis

**Conclusions:** Our study shows that the new mobile phone based app scoring method is effective in bringing analytical validity without losing clinical validity and may be a good alternative for those laboratories lacking the DIA software.

## 273 BAP1 Loss in Triple Negative Breast Cancer

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**Disclosures:** Theodore Vougiouklakis: None; Christopher Schwartz: None; Paolo Cotzia: None; Matija Snuderl: None; George Jour: None; Farbod Darvishian: None

**Background:** *BAP1* (BRCA1-Associated Protein 1) is a tumor suppressor gene, which exerts its effect through chromatin modulation, transcriptional regulation and DNA damage response pathway. *BAP1* inactivation can occur through chromosomal deletions involving its locus (3P21.1) or due to mutations. *BAP1* alterations can be detected at the protein level by demonstrating nuclear loss of staining on immunohistochemistry (IHC). It has been shown that the absence of nuclear BAP1 staining on IHC correlates with biallelic genomic loss. A rare association between BAP1 mutation and breast cancer (<1%) has been reported. We sought to evaluate BAP1 loss in triple negative breast cancer (TNBC) by IHC and gene sequencing.

**Design:** Three tissue microarray (TMA) blocks of TNBC were used for IHC. IHC for BAP1 was performed using BAP1 antibody (C-4: Santa Cruz, 1:100). For sequencing, a section of the tumor was compared with corresponding matched normal tissue. Macrodissection for tumor enrichment was performed. DNA was subjected to deep sequencing (average 500x) using our customized panel targeting all exonic and certain select intronic areas in 580 cancer related genes (NGS580) then analyzed using our own bioinformatics pipeline including MuTect2 and Low Freq for single-nucleotide and small indel somatic variants (SNV; indels). Control-FREEC was used for detection of copy number alterations (CNA).

**Results:** The TMA blocks contained 101 cases of TNBC as follows: apocrine carcinoma (9), invasive lobular carcinoma (3), adenoid cystic carcinoma (1), metaplastic carcinoma (5) and no specific histologic type (NST, 83). Only one case of TNBC-NST showed complete loss of BAP1 nuclear staining. The tumor with BAP1 loss was grade 3 with unusual squamoid features and unusual spindle cell stroma. The tumor and the stroma were negative for p63. No BAP1 mutation or copy number loss was identified by NGS580. Instead, we detected mutations in *TP53* (SNV c.G422T), *ATR* (SNV, c.G5149A), *ERCC3* (SNV, c.G995C), *ROS1* (splicing) and *TLX1* (splicing). Conversely, there was another case of TNBC-NST that showed BAP1 mutations (SNV, c.G176A) on NGS5480 but no protein loss on IHC.

**Conclusions:** Approximately 1% of our TNBC cohort showed loss of BAP1 on IHC in agreement with the literature. No copy number alteration or mutation was detected in the *BAP1* gene by NGS580. These findings suggest that the loss of BAP1 protein is mediated by alternative epigenetic mechanisms affecting mRNA splicing.

## 274 Effect of EDTA Decalcification on ER and PR Immunohistochemistry and HER2/neu Fluorescence In Situ Hybridization in Breast Carcinoma

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**Disclosures:** Erik Washburn: None; Xiaoyu Tang: None; Carla Caruso: None; Michelle Walls: None; Bing Han: None

**Background:** Bone is the most common site of metastasis in breast carcinoma (BC). Treatments for metastatic BC include endocrine therapy or HER2 active agents depending on the tumor's receptor status. Histology of bone biopsies requires decalcification which may affect immunostochemical (IHC) assessment of estrogen receptor (ER) and progesterone receptor (PR) as well as in situ hybridization (FISH) studies of HER2. EDTA based decalcifying solutions have been theorized to have no significant impact on ER and PR IHC or HER2 FISH studies. We completed a prospective study of the effect of EDTA decalcification on ER and PR IHC and HER2 FISH in 31 cases of BC. To our knowledge, this is the most extensive study of the effect of EDTA on ER/PR IHC and HER2 FISH in BC.

**Design:** Samples from 31 BC resections were prosectively collected and formalin fixed between 12-24 hours. Control samples were routinely processed while test samples were placed in EDTA for 48 hours, and then processed. ER and PR slides were blinded, randomized, and evaluated by two investigators (EW and XT). Tumor cell staining was estimated from 0-100%. Sections underwent HER2 FISH assays using Her-2 and CEP17 probes. 25 tumor cell nuclei were assessed via fluorescent microscope by a certified technologist (MW). An average HER2 copy number and HER2/CEP17 ratio was calculated (see Table 1). ER and PR positivity, average HER2 copy number, and HER2/CEP17 ratios underwent statistical analysis using IBM SPSS 25. Mean and median paired differences between EDTA and control samples were compared using paired-samples T tests (PST) (P-values < 0.05 considered significant) and Wilcoxon signed-rank tests (WSR) (Z values < -1.96 or > 1.96 considered significant).

**Results:** PST tests yielded no significant difference between EDTA and control tissue for ER% (P=0.697), PR% (P=0.907) (illustrated in Fig. 1), HER2 copy number (P=0.094), and HER2/CEP17 ratio (P=0.396) (illustrated in Fig.2). WSR tests yielded no significant difference between EDTA and control tissue for ER% (Z=0.547), PR% (Z=0.088), HER2 copy number (Z=1.226), and HER2/CEP17 ratio (Z=1.730).

Table 2. Estrogen receptor (ER) and progesterone receptor (PR) nuclear positivity via immuno-histochemical stains (IHC) and HER2 copy number and HER2/CEP17 ratios via fluorescence in situ hybridization (FISH) in 31 paired samples of breast cancer routinely processed (control) and after decalcification in EDTA for 48 hours (EDTA)

| Patient | ER (IHC) | | PR (IHC) | | HER2 Copy # (FISH) | | HER2/CEP17 (FISH) | |
|---|---|---|---|---|---|---|---|---|
| | ER Control% Positive | ER EDTA% Positive | PR Control% Positive | PR EDTA% Positive | Control HER2 Copy # | EDTA HER2 Copy # | Control HER2/CEP17 | EDTA HER2/CEP17 |
| 1 | 90 | 95 | 60 | 60 | 1.52 | 1.44 | 1.19 | 1.03 |
| 2 | 90 | 90 | 70 | 80 | 1.48 | 1.52 | 1.06 | 1.06 |
| 3 | 80 | 70 | 70 | 70 | 1.44 | 1.28 | 0.88 | 0.82 |
| 4 | 90 | 80 | 90 | 80 | 1.68 | 1.44 | 1.24 | 1.06 |
| 5 | 90 | 95 | 40 | 50 | 1.76 | 1.44 | 0.83 | 1.06 |
| 6 | 95 | 90 | 95 | 90 | 1.6 | 1.32 | 0.95 | 0.94 |
| 7 | 90 | 90 | 90 | 70 | 2.92 | 1.8 | 1.92 | 1.05 |
| 8 | 95 | 95 | 1 | 5 | 1.44 | 1.8 | 1.08 | 1.5 |
| 9 | 95 | 95 | 40 | 40 | 1.84 | 1.6 | 1.73 | 1.08 |
| 10 | 90 | 90 | 0 | 0 | 4.24 | 4.12 | 2.4 | 3.81 |
| 11 | 80 | 80 | 50 | 50 | 1.44 | 1.64 | 1.13 | 1.21 |
| 12 | 80 | 70 | 10 | 5 | 2.05 | 2.12 | 1.03 | 1.1 |
| 13 | 95 | 95 | 5 | 1 | 1.85 | 1.95 | 1.47 | 1.52 |
| 14 | 90 | 95 | 5 | 1 | 1.56 | 1.52 | 1.08 | 1.15 |
| 15 | 95 | 95 | 50 | 50 | 2.08 | 2.16 | 1.37 | 1.64 |
| 16 | 0 | 0 | 0 | 0 | 1.64 | 1.52 | 1.05 | 1 |
| 17 | 95 | 95 | 90 | 90 | 1.16 | 1.6 | 0.88 | 0.95 |
| 18 | 0 | 0 | 0 | 0 | 8.6 | 8.32 | 6.14 | 6.5 |
| 19 | 0 | 0 | 0 | 0 | 1.96 | 1.64 | 0.71 | 0.73 |
| 20 | 0 | 0 | 0 | 0 | 10 | 11.06 | 8.62 | 10.74 |
| 21 | 95 | 90 | 0 | 0 | 1.16 | 1.48 | 0.9 | 1.03 |
| 22 | 95 | 95 | 90 | 80 | 2.15 | 2.24 | 1.29 | 1.54 |
| 23 | 80 | 95 | 70 | 95 | 2.44 | 2.4 | 1.27 | 1.3 |
| 24 | 90 | 95 | 20 | 30 | 1.52 | 1.64 | 1.09 | 1.21 |
| 25 | 90 | 90 | 50 | 60 | 1.6 | 1.72 | 1.1 | 1.1 |
| 26 | 0 | 0 | 0 | 0 | 1.92 | 1.36 | 0.98 | 1.03 |
| 27 | 95 | 95 | 5 | 1 | 5.28 | 4.76 | 2.24 | 2.16 |
| 28 | 0 | 0 | 0 | 0 | 1.36 | 1.36 | 0.67 | 1 |
| 29 | 70 | 95 | 50 | 40 | 1.51 | 1.72 | 1.11 | 1.23 |
| 30 | 95 | 95 | 90 | 90 | 9.8 | 9 | 4.71 | 6.34 |
| 31 | 80 | 80 | 20 | 5 | 7.48 | 6.36 | 5.44 | 4.06 |

Figure 1 - 274

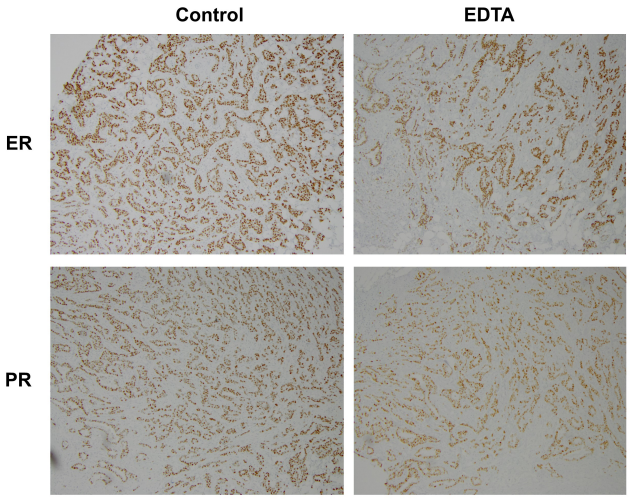


Figure 1. Estrogen (ER) and Progesterone (PR) receptor immunohistochemical stains of control tissue and EDTA decalcified tissue samples of invasive ductal carcinoma (Patient #2). (200X magnification)

Figure 2 - 274

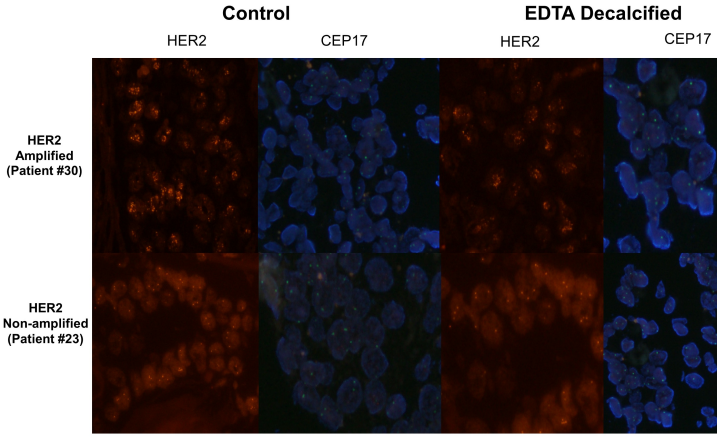


Figure 2. Her2 (orange) and CEP17 (green) fluorescence in situ hybridization images of control and EDTA decalcified tissue samples from selected examples of HER2 amplified invasive ductal carcinoma (Patient #31) and HER2 non-amplified invasive ductal carcinoma (Patient #23). (400X magnification)

**Conclusions:** Our study is the largest prospective evaluation of EDTA effect on ER/PR IHC and HER2 FISH assays. The results show EDTA induces no relevant change in the immunopositivity of ER/PR and has no significant effect on HER2 FISH. Use of EDTA in bony tissue samples is therefore a valid decalcification method to ensure accurate assessments of ER and PR IHC and HER2 FISH in metastatic BC.

## 275 Impact of the 2018 ASCO/CAP HER2 Guideline Updates on HER2 Assessment in Breast Cancer with HER2 Immunohistochemistry Equivocal Results

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**Disclosures:** Hannah Wen: None; Katia Ventura: None; Jin Xu: None; Dara Ross: None; Chau Dang: *Grant or Research Support*, Roche/Genentech; *Grant or Research Support*, PUMA; Mark Robson: None; Larry Norton: None; Monica Morrow: None; Edi Brogi: None

**Background:** The ASCO/CAP guidelines for HER2 testing in breast cancer were updated in 2018 (2018 ASCO/CAP) to address clinical questions related to the 2013 guidelines (2013 ASCO/CAP), mainly focused on the interpretation of uncommon results using dual-probe fluorescent in situ hybridization (FISH). Herein, we assessed the impact of the 2018 ASCO/CAP on the HER2 status in breast cancers with equivocal HER2 immunohistochemistry (IHC) results.

**Design:** At our center, we routinely assess HER2 expression by IHC in all primary, recurrent and metastatic breast cancers. All HER2 IHC equivocal (2+ or 1+ to 2+) cases are reflexed to HER2 FISH. We retrospectively reviewed the HER2/CEP17 ratio (ratio) and HER2 copy number (CN) of HER2 IHC equivocal breast cancers tested at our center between 01/2014 and 05/2018. The HER2 results using the 2013 and 2018 guidelines were compared.

**Results:** A total of 1666 HER2 FISH reports from 1421 patients were identified. Based on the 2013 ASCO/CAP, HER2 FISH was positive in 346 (21%) cases, equivocal in 242 (14.5%), and negative in 1078 (65%). Table 1 summarizes the classification of HER2 results using the 2013 and 2018 ASCO/CAP. A total of 258 (15%) cases were classified differently using the 2018 ASCO/CAP, including 242 (14.5%) previously “equivocal” cases (ratio <2, HER2 CN ?4 and <6) reclassified as negative, and 16 (1%) previously positive cases with ratio ?2 but HER2 CN <4 reclassified as negative. The previously “equivocal” group was not treated with anti-HER2 therapy. Patients with ratio ?2 but HER2 CN <4 and with ratio <2 but HER2 CN ?6 received HER2-targeted therapy, including 4 in the neoadjuvant setting and did not have pathologic complete response.

Table 1. Comparison of HER2 dual-probe FISH results in breast cancer with equivocal HER2 IHC using 2013 and 2018 ASCO/CAP guidelines

| 2013 guidelines<br><br>n (%) | 2018 guidelines, n (%) | | | | |
|---|---|---|---|---|---|
| | Group 1<br><br>Ratio?2, HER2 CN ?4 | Group 2<br><br>Ratio ?2<br><br>HER2 CN <4 | Group 3<br><br>Ratio <2<br><br>HER2 CN ?6 | Group 4<br><br>Ratio <2<br><br>HER2 CN ?4, <6 | Group 5<br><br>Ratio <2<br><br>HER2 CN <4 |
| | Positive | Negative | Positive | Negative | Negative |
| Positive<br><br>346 (21%) | 318 (19%) | 16 (1%) | 12 (0.7%) | 0 | 0 |
| Equivocal<br><br>242 (14.5%) | 0 | 0 | 0 | 242 (14.5%) | 0 |
| Negative<br><br>1078 (65%) | 0 | 0 | 0 | 0 | 1078 (65%) |
| Total<br><br>1666 (100%) | 318 (19%) | 16 (1%) | 12 (0.7%) | 242 (14.5%) | 1078 (65%) |

**Conclusions:** Cases with HER2/CEP 17 ratio <2, HER2 CN ?4 and <6 accounted for 14.5% in our cohort. The change of classification from "equivocal" to "negative" using the 2018 ASCO/CAP had no clinical implications since these patients were not treated with anti-HER2 therapy at our center. Cases with ratio ?2 *and* HER2 CN <4 or ratio <2 *and* HER2 CN ?6 accounted for a small percentage (1.7%) of all cases in our cohort and the numbers are insufficient for evaluation of HER2-targeted therapy benefit.

## 276 Clinical, Radiological and Pathological Features of Atypical Ductal Hyperplasia Predicting Pathologist Disagreement

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**Disclosures:** Willard Wong: None; Kiran Jakate: None; Hala Faragalla: None; Fang-I Lu: None

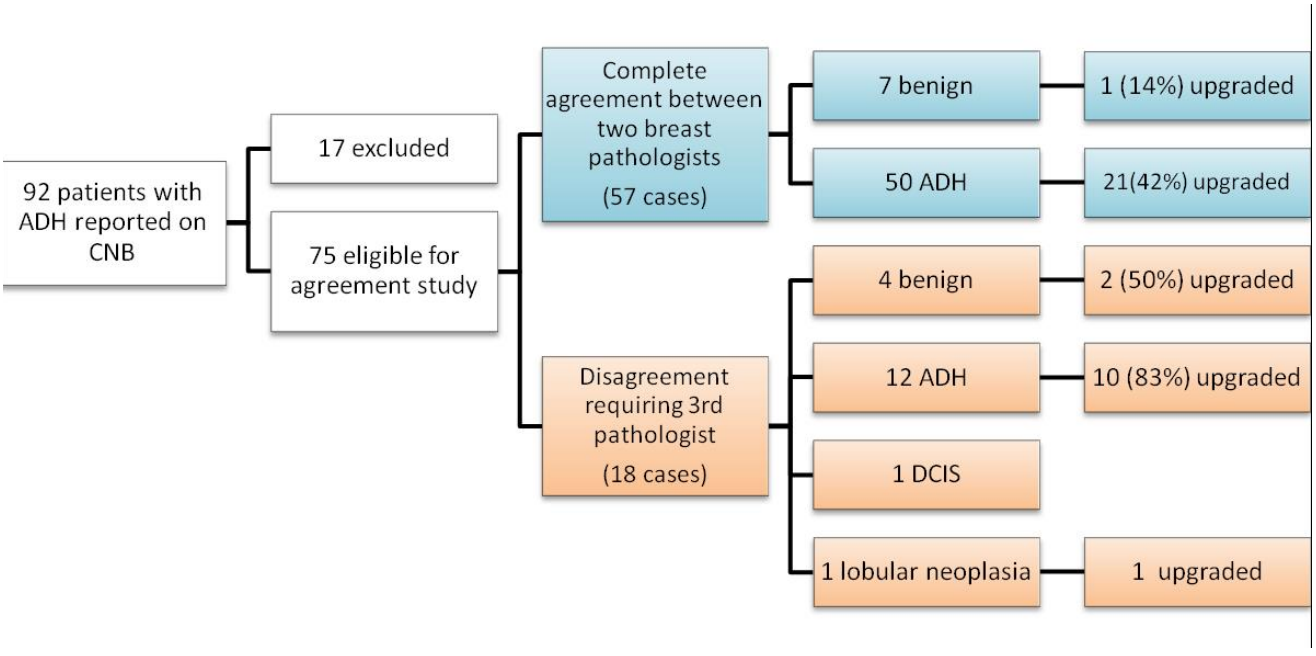
**Background:** Atypical ductal hyperplasia (ADH) is a non-malignant breast lesion frequently diagnosed on core needle biopsy (CNB). Routine management consists of surgical excision (SE); however, upgrade on SE to ductal carcinoma in situ (DCIS) or invasive carcinoma (IC) is encountered in about 15-30% of cases and may necessitate additional surgery. Given these implications for management, a correct diagnosis of ADH is imperative. As such, we studied inter-pathologist agreement of ADH on CNB as well as clinical, radiologic and histologic features associated with disagreement.

**Design:** Our hospital LIS (CoPath) was retrospectively searched for ADH CNB diagnoses from 2010-16. CNB without subsequent SE and cases with concurrent DCIS or IC within the same breast quadrant were excluded. Diagnostic slides from eligible cases were independently reviewed by 2 breast pathologists (blinded to the SE diagnosis), to determine the presence of ADH. Cases eliciting disagreement between the 2 reviewing pathologists were evaluated by a 3rd breast pathologist to achieve diagnosis by consensus. Clinical, radiological and pathological features were compared between cases with complete diagnostic agreement versus disagreement. The reported SE diagnosis was used to determine upgrade.

**Results:** Ninety-two patients were identified with an initial diagnosis of ADH on CNB. Of these, 17 were excluded (1 IC within same quadrant, 7 without SE, 9 not available for review). Seventy-five cases were evaluated and the complete agreement rate was 76.0% (n=57; Figure 1). Cases with disagreement (n=18) showed association with upgrade on SE, fewer cores retrieved, and ultrasound-guided biopsy (p=0.016, 0.029 and 0.001, respectively; Table 1). Sixty-two cases were confirmed as ADH by reviewing pathologists. A subanalysis of ADH cases alone is also shown in Table 1: disagreement was again associated with upgrade as was fewer cores retrieved. Additional factors associated with disagreement included calcifications absent in atypia, greater linear extent of atypia, and greater percent of cores involved by atypia (p=<0.001, 0.044, and 0.013, respectively

| Table 1. Analysis of histologic, radiologic and clinical features of all cases | | | |
|---|---|---|---|
| Histological Features | Agreement n=57 (%) | Disagreement n=18 (%) | p |
| Number upgraded on excision | 22 (38.6) | 13 (72.2) | 0.016 |
| Number not upgraded on excision | 35 (61.4) | 5 (27.8) | |
| # of cores retrieved | 6.54 | 4.89 | 0.029 |
| Radiological Features | | | |
| Imaging Modality Used for Core Biopsy: | | | |
| US | 11 (19.3) | 10 (55.6) | 0.001 |
| Stereo | 46 (80.7) | 7 (38.9) | |
| MRI | 0 | 1 (5.6) | |
| Clinical Features | | | |
| Age (years) | 56.83 | 53.83 | 0.287 |
| Personal History of Breast Cancer | | | |
| Absent | 55 (96.5) | 16 (88.9) | 0.242 |
| Present | 2 (3.5) | 2 (11.1) | |
| Breast/Ovarian Cancer in 1st Degree Relative | | | |
| Absent | 50 (87.7) | 14 (77.8) | 0.444 |
| Present | 7 (12.3) | 4 (22.2) | |
| Prior Chest Irradiation | | | |
| Absent | 56 (98.2) | 16 (88.9) | 0.141 |
| Present | 1 (1.8) | 2 (11.1) | |
| History of HRT use | | | |
| Absent | 46 (80.7) | 17 (94.4) | 0.273 |
| Present | 11 (19.3) | 1 (5.6) | |
| **Analysis of histologic, radiologic and clinical features of ADH cases** | | | |
| Histological Features | Agreement n=50 (%) | Disagreement n=12 (%) | p |
| ADH | 48 (96.0) | 12 | 1 |
| At least ADH bordering on DCIS | 2 (4.0) | 0 | |
| Number of ADH upgraded on excision | 21 (42.0) | 10 (83.3) | 0.022 |
| Number of ADH not upgraded on excision | 29 (58.0) | 2 (16.7) | |
| Calcifications present in atypia | 40 (80.0) | 2 (16.7) | 0.0001 |
| Calcifications absent in atypia | 10 (20.0) | 10 (83.3) | |
| Extent of atypia (mm) | 1.67 | 2.39 | 0.044 |
| # cores involved by atypia | 2.08 | 1.83 | 0.527 |
| # TDLU involved by atypia | 2.18 | 2 | 0.624 |
| # of cores retrieved | 6.74 | 4.92 | 0.045 |
| % cores involved by atypia | 0.34 | 0.53 | 0.013 |

Figure 1 - 276




**Conclusions:** Our results show an important association between inter-pathologist disagreement of an ADH diagnosis on CNB and upgrade at the time of SE. A consensus diagnostic approach should be considered in some cases, possibly to include those which are ultrasound guided (often for a mass lesion) and with a limited number of cores.

## 277 Alteration of biomarker status during metastatic progression and its prognostic value in patients with metastatic breast cancer

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**Disclosures:** Ji Won Woo: None; Yul Ri Chung: None; Hyun Jeong Kim: None; Soomin Ahn: None; So Yeon Park: None

**Background:** Tissue confirmation of metastatic breast cancer (MBC) is still optional and is not routinely performed. However, there are cumulating evidences that alteration of biomarker status occurs frequently and has an effect on treatment response. The purpose of this study was to evaluate the frequency of biomarker alteration and to clarify its possible impacts on prognosis of patients with MBC.

**Design:** A total of 152 patients with MBC at the time of initial diagnosis or during follow up after operation were included in this study. Alteration of biomarker status including estrogen receptor (ER), progesterone receptor (PR), HER2 and Ki-67 in MBCs, its frequencies according to different metastatic sites, and its association with patients' survival were analyzed.

**Results:** The expression levels of ER and PR by percentage of positive tumor cells and Allred score decreased significantly in MBCs compared to those in primary tumors. ER, PR, HER2 and Ki-67 status changed in 9 (6.0%), 40 (26.3%), 15 (9.9%), and 29 (19.1%) patients, respectively. ER, PR and HER2 mainly showed positive to negative conversion, whereas Ki-67 mostly revealed changes from low (less than 20%) to high index (20% or more). Among various metastatic sites, biomarker status change occurred slightly more often in metastases to liver than in those to other sites. Time to progression was shorter in tumors showing negative conversion in hormone receptor status, compared to persistent hormone receptor-positive tumors. In survival analyses, positive to negative conversion of ER in MBC was associated with worse overall survival of the patients, compared to persistent positive tumors. However, alteration in PR, HER2 and Ki-67 index had no prognostic significance.

**Conclusions:** Alteration of biomarker status in breast cancer metastases is not a rare phenomenon, and usually occurs in bad direction. Especially, negative conversion of ER status could predict worse prognosis. Thus, it would be beneficial to evaluate the biomarker status of MBC not only for determination of treatment option, but also for prognostication of the patients.

## 278 High Concordance in HER2 Status Between Core Biopsy and Excisional Specimens

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**Disclosures:** Jessie Wu: None; Martin Chang: None

**Background:** HER2 is a prognostic and predictive biomarker in breast cancer, and is determined on all cases of invasive breast carcinoma. It is standard practice to perform the first test on a diagnostic core biopsy. The 2013/2018 statements of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) expert panel describe situations in which HER2 **may** be re-tested on an excisional specimen at pathologists' discretion. Our goal was to audit a consecutive series of cases in which HER2 was determined in both core and excisional specimens.

**Design:** In accordance with the protocol approved by our hospital's Research Ethics Board, records of all HER2 tests in a 2-year period (2016-2017) were retrieved. Cases in which the same patient was evaluated for HER2 in the same tumor for both core and excisional specimens were identified. In our reference lab, the decision to re-test excisional specimens varies with pathologist. HER2 status was reclassified according to the 2018 ASCO/CAP update and discordant cases audited for tumor characteristics.

**Results:** A total of 270 patients with invasive breast carcinoma, all with HER2 tested on both core and excisional sampling of the same tumor. In the core specimens, HER2 IHC was positive in 15 (5.5%), negative in 167 (62%), and equivocal in 88 (32.5%) cases. FISH was performed in 88, all having 2+ IHC score. HER2 FISH was positive in 11/88 (13%), negative in 74/88 (84%), and indeterminate/heterogeneous in 3/88 (3%). In the excisional specimens, HER2 IHC was positive in 17 (6%), negative in 162 (60%), and equivocal in 91 (34%) cases. FISH was performed in 91 IHC-2+ cases and 1 IHC-1+ case. HER2 FISH was positive in 9/92 (10%), negative in 78/92 (85%), and indeterminate/heterogeneous in 5/92 (5%).

The final HER2 result was concordant between core and excision in 256/270 (94.8%) cases. In an additional 4/270 cases (1.5%), there was heterogeneity in one or more specimens resulting in a non-clinically significant difference. In the remaining 10/270 (3.7%, Table 1) discordant cases, the plurality were Grade 3 and all discordances resulted from either borderline HER2 FISH (7/10) or heterogeneity (3/10).

**Table 1: Audit of Cases with Clinically Significant Discordance in HER2 (Core vs Excision)**

| Discordant Case | Core HER2 Result (IHC/FISH Combined) | Core FISH Ratio (HER2/CEP17) | Excision HER2 Result (IHC/FISH Combined) | Excision FISH Ratio (HER2/CEP17) | Tumor Grade | Heterogeneity | ER/PgR Result (+ or -) |
|---|---|---|---|---|---|---|---|
| 1 | Negative | N/A | Heterogeneous | 2.10/2.03: 90%<br>8.05/2.43: 10% | 2 | Yes (10%) | +/+ |
| 2 | Negative | N/A | Positive | 5.48/2.72 (Borderline*) | 1 | No | +/+ |
| 3 | Negative | 3.00/2.18 | Positive | 5.90/2.80 (Borderline*) | 3 | No | +/- |
| 4 | Negative | 3.05/2.58 | Heterogeneous | 3.80/2.73: 75%<br>6.80/5.08: 25% | 2 | Yes (25%) | +/+ |
| 5 | Negative | 4.36/2.33 (Borderline*) | Positive | 8.63/3.30 | 3 | No | +/+ |
| 6 | Negative | 4.00/2.28 (Borderline*) | Positive | 6.63/2.55 | 2 | No | +/+ |
| 7 | Positive | 6.30/2.83 | Heterogeneous | 4.13/3.25 (scattered heterogeneity) | 2 | Yes (scattered heterogeneity) | +/+ |
| 8 | Positive | 4.15/1.43 (monosomy/ borderline*) | Negative | N/A | 3 | No | +/+ |
| 9 | Positive | 6.49/4.09 | Negative | 4.74/3.74 (Borderline*) | 3 | No | +/+ |
| 10 | Positive | 6.33/3.4 | Negative | 4.15/2.62 (Borderline*) | 3 | No | +/+ |

*Borderline HER2 FISH defined as HER2 copies between 4 and 6 in either core or excision.

**Conclusions:** HER2 results on core biopsy were concordant with the excisional specimen in the vast majority (96.3%) of cases. Discordances tended to be Grade 3, or borderline/heterogeneous on FISH. These results support the ASCO/CAP recommendation to retest selectively, rather than routinely, on an excisional specimen.

## 279 Bcl-2 Expression Correlates with Oncotype DX Recurrence Score in Mammary Carcinoma

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**Disclosures:** Yan Xiang: None; Li Li: None; J. Steve Hou: None; Fernando Garcia: None; Kareem Mohammed: None; Mark Zarella: None

**Background:** B-cell lymphoma 2 (Bcl-2) proto-oncogene alterations are involved in tumor genesis and play a vital role in regulating cell apoptosis. Although Bcl-2 protein expression has been suggested as a candidate prognostic factor for breast cancer, its use to predict the risk of breast cancer recurrence has not been determined.

**Design:** This study aimed to investigate the correlation between Bcl-2 expression and the Oncotype DX recurrence score (RS), commonly used to assess the likelihood of breast cancer recurrence and chemotherapy benefit in hormone receptor-positive node-negative patients. RS is measured by RT-PCR to analyze the expression of 21 genes: 16 cancer-related genes and five reference genes. The Bcl-2 H-score obtained using Leica Imaging System was correlated with the RS score. 158 breast carcinoma cases eligible for RS testing were collected from our university's breast repository. ANOVA Test, Pearson's correlation and Student's t-test were performed for statistic analysis.

**Results:** Most tumors showed heterogeneous Bcl-2 staining. Notably, low RS (<25, n=133) was correlated with high Bcl-2 H-score (P=0.0003, r=-0.4). We compared the correlation with RS when Bcl-2 was score manually and with the H-score of Bcl-2 obtained with image analysis. H-score showed a better correlation with RS than when Bcl-2 was scored manually using percent positive cells, suggesting that automated image analysis is best for scoring Bcl-2. We further demonstrated that Bcl-2 H-score provided additional information for the prediction of RS not wholly captured by ER, PR, Ki-67, and Her2 expression, suggesting independent prognostic utility.

**Conclusions:** 1) Bcl-2 H score was observed to have a significant inversed correlation with the Oncotype DX RS; 2) Bcl-2 IHC scored using automated image analysis(multiplied by staining intensity and percentage of positive cells) shows better correlation with RS than percentage of positive cells; 3) Bcl-2 may serve as a viable alternative in underprivileged areas to more expensive gene expression analysis approaches; 4) The results of our study establish a possible quantitative framework for interpreting Bcl-2 expression for predicting breast cancer recurrence likelihood.

## 280 Clinicopathologic Features of Breast Cancers with HER2/CEP17 Ratio <2, HER2 Copy Number ≥4 and <6 Signals/Cell

Jin Xu[1], Edi Brogi[1], Katia Ventura[1], Dara Ross[1], Chau Dang[1], Mark Robson[1], Larry Norton[2], Monica Morrow[1], Hannah Wen[1]
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Chau Dang: *Grant or Research Support*, Roche/Genentech; *Grant or Research Support*, PUMA; Mark Robson: None; Larry Norton: None; Monica Morrow: None; Hannah Wen: None

**Background:** Breast cancer with *HER2*/CEP17 ratio (ratio) <2, *HER2* copy number (CN) ≥4 and <6 signals/cell was defined as "equivocal" according to the 2013 ASCO/CAP guidelines. The 2018 ASCO/CAP guidelines classified this group as HER2 negative in the absence of HER2 protein overexpression (3+). We reviewed the frequency and clinicopathologic features of such cases.

**Design:** At our center, we routinely assess HER2 by immunohistochemistry (IHC) in all primary, recurrent and metastatic breast cancers (BCs). All HER2 IHC equivocal (2+ or 1+ to 2+) cases are reflexed to HER2 dual-probe fluorescent in situ hybridization (FISH). We retrospectively reviewed the HER2 FISH results between 01/2014 and 05/2018, and identified all BCs with ratio <2 and *HER2* CN ≥4 and <6.

**Results:** Of 1421 BCs with equivocal HER2 IHC and reflex FISH testing, 125 (9%) had ratio<2, and *HER2* CN ≥4 and <6. Our study cohort included only untreated primary BCs in this group (n=103). Patient median age was 60 years (25-89). Median tumor size was 1.7 cm (0.2-4.6). The most common tumor type was invasive ductal carcinoma NOS (72; 70%), followed by invasive micropapillary carcinoma or with micropapillary features (21; 20%). Thirty-seven (36%) patients had node positive disease. Most BCs were positive for ER (96; 93%) and/or PR (82; 80%). The 21-gene recurrence score (RS) was assessed in 42 BCs, with a median RS of 22 (5-45). RS was ≤25 in 27 and >25 in 15 BCs. Comparing to 226 primary BCs with equivocal HER2 IHC and FISH ratio ≥2, *HER2* CN ≥4/cell (FISH positive) in the same study period, BCs with ratio <2 and *HER2* CN ≥4 and <6 were significantly associated with slightly older age (mean age 59 vs 55 years; p=0.02) and higher incidence of micropapillary histology (20% vs 8%; p=0.001). There was no significant difference in ER (93% vs 87%; p=0.13) or PR (80% vs 72%; p=0.14) positivity between the two groups. Most patients with ratio <2 and *HER2* CN ≥4 and <6 did not receive anti-HER2 therapy. Fifty-six (54%) patients received chemotherapy and 81 (78%) had adjuvant endocrine therapy. At a median follow-up of 25 months (range 1-57), 95% patients had no evidence of disease.

**Conclusions:** Most breast cancers with HER2 equivocal IHC, and *HER2*/CEP17 ratio <2, *HER2* CN ≥4 and <6 signals/cell in our cohort were hormone receptor positive. Micropapillary morphology was common. Most patients did not receive anti-HER2 therapy, and remained disease-free after initial treatment, although the follow-up period was relatively short.

## 281 Low Expression of Tumor Suppressor DEAR1 in the Triple Negative Breast Cancer Correlated with Young Age and Early Metastasis

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**Disclosures:** Fei Yang: None; Uyen Le: None; Nanyue Chen: None; Seetharaman Balasenthil: None; Barbara Mino: None; Suyu Liu: None; Maria Rubin: None; Aysegul Sahin: None; Ignacio Wistuba: None; Ann Killary: None

**Background:** Triple negative breast cancer is a highly aggressive breast cancer with a high risk of metastases. Recent studies point to the role of breast cancer stem cells thought to arise from EMT in driving TNBC dissemination and chemo-resistance. DEAR1, a tumor suppressor gene encoding a TRIM subfamily member, has been defined an important role in the regulation of acinar morphogenesis, cell polarity and as a negative regulator of EMT. Therefore we investigated whether DEAR1 expression could serve as a biomarker for risk of metastasis in TNBC.

**Design:** FFPE materials from 153 surgically resected breast cancer specimens at MD Anderson Cancer Center were used to build a tissue microarray, including 103 TNBC and 50 non TNBC. IHC was performed on Leica BOND-MAX with DEAR1antibody provided by Bethyl Lab. DEAR1 expression on both cytoplasm and membrane were evaluated using Aperio separately. H-scores multiplied by the intensity (from 1 to 3) and percentage of positive tumor cells (from 1-100%) were correlated with clinic pathologic features and follow up data.

**Results:** Malignant cells cytoplasmic DEAR1 H-score greater than 10 was detected in 71.8% (74/103) of TNBC and membrane H-score greater than 10 was detected in 64% (66/103). When DEAR1 expression on cytoplasm was dichotomized by the median or categorized by tertiles, we observed a significant association between low DEAR1 and younger age of diagnosis (p=0.04 and p=0.01, respectively) in the TNBC. When age was dichotomized by younger than 50 yrs of age *vs* 50 yrs and older (p=0.04), we continue to see a significant correlation between low DEAR1 expression and early-onset breast cancer in the TNBC. Although cytoplasmic and membrane DEAR1 H-scores were strongly correlated (rho=0.88, p<0.01), statistically significant differences observed for age among DEAR1 cytoplasmic expression were not detected among DEAR1 membrane expression. We also observed a borderline significantly correlation between low

DEAR1 expression and shorter time to metastasis (p=0.05, HR=0.41) in TNBC patients. The risk of developing metastasis is 59% less for patients with high DEAR1 cytoplasmic expression compared to patients with low expression.

**Conclusions:** DEAR1 could potentially serve as a biomarker to stratify early onset TNBC patients for targeted stem cell therapies aimed at the pathways regulated by DEAR1.

## 282 AI Empowered High-accuracy Prognosis for Triple-Negative Breast Cancer using Quantitative Multiplex Immunofluorescence Images

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**Disclosures:** Joe Yeong: None; Frank Guan: None; Gui Yan: None; Jabed Iqbal: None; Aye Aye Thike: None; Yiyu Cai: None

**Background:** We aim to develop a novel methodology towards accurately predicting disease-free survival for patients with triple-negative breast cancer through analysing our recently reported quantitative multiplex immunofluorescence (QmIF) images with Artificial Intelligence techniques particularly deep learning.

**Design:** There are four steps in the methodology: Data Collection and Preparation, Neural Network Training, Validation, and Application. In the first step, two different types of data, including disease-free survival data and multiplex immunofluorescence image data, were collected from a group of patients with triple-negative breast cancer diagnosed between 2003 and 2013 in Singapore General Hospital which were stained simultaneously with 6 markers (PD-L1, PD-1, CD68, CD8, Foxp3 and Cytokeratin) by using QmIF, representing the training dataset. Next, the training dataset acted as input to train a neural network with algorithms to derive the relationship between detected features from the immunofluorescence images and the survival data of patients. Similar data from another group of patients (testing dataset) were used to validate and improve the trained neural network. Upon completion of the neural network training, disease-free survivals of new patients were predicted by analysing their breast cancer immunofluorescence image data with the trained neural network as a data-driven approach.

**Results:** The medical and breast cancer immunofluorescence image data for 92 patients were used as training data, and the dataset for another 8 patients were used as testing data. With the trained neural network, >=96% evaluation accuracy was achieved on the testing dataset.

**Conclusions:** Our results show that by analysing the multiplex immunofluorescence image data with deep learning techniques, disease-free survivals of patients with triple negative breast cancer can be predicted at high accuracy. The systematic methodology developed can be further extended to enhance diagnosis and prognosis for other diseases. Further studies are warranted on a larger cohort.

## 283 Mucocele-Like Lesions of the Breast on Core Needle Biopsy: an Institutional review and Meta-Analysis of the Literature

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**Disclosures:** Lourdes Ylagan: None; Kimberly Allison: None; Debra Ikeda: *Consultant*, Hologic inc

**Background:** Mucocele-like lesions (MLL) are rare lesions of the breast (<1%) which because of its rarity and frequent association with known risk lesions of the breast are frequently removed surgically. The goal of this study is to present our data of the last 10 years and do a meta-analysis of the literature on this topic and present evidence for conservative management of MLL without associated atypia.

**Design:** A strict definition for pure MLL is used (Figure 1 and 2), presence or absence of atypia is reviewed by 2 subspecialty breast pathologists and a strict definition of what constitutes an *Upgrade* to carcinoma is followed. A search in our institutions' data files for MLL from 2008-2018 was done. A concomitant search of the literature focusing on upgrade rate of MLL with and without atypia over the last 20 years was performed.

**Results:** Our meta-analysis indicates that pure MLLs without atypia over the past 20 years (n= 516) had an upgrade rate of 4.6%, with atypia (n=144) an upgrade rate of 17.3%. Our institutional upgrade rate to carcinoma for MLLs with and without atypia is 11% and 0%, respectively.

**Table 1. Meta-analysis of Mucocele-Like Lesions on core needle biopsies with and without atypia**

| Year Published | Author | Excised | Without Atypia | Upgrade toDCIS | Upgrade toIMC | With Atypia | Upgrade to DCIS | Upgrade to IMC | Total Upgrade |
|---|---|---|---|---|---|---|---|---|---|
| Current | Ylagan, L | 42 | 6 | 0 (0%) | 0 (0%) | 36 | 2 (5%) | 2 (5%) | 4/36=11% |
| 2017 | Zhang,G | 28 | 19 | 1 (5%) | 0 (0%) | 9 | 0 (0%) | 3 (30%) | 4/28=14% |
| 2017 | Dash, I | 103 | 103 | 5 (5%) | 0 (0%) | NA | NA | NA | 5/103=5% |
| 2016 | Meares,A | 102 | 75 | 0 (0%) | 9 (12%) | 27 | 0 (0%) | 4 (15%) | 13/102=13% |
| 2016 | Gibreel W | 29 | 15 | 0 (0%) | 1 (7%) | 14 | 0 (0%) | 0 (0%) | 1/29= 3.4% |
| 2015 | Park, YJ | 27 | 21 | 0 (0%) | 0 (0%) | NA | NA | NA | 0% |
| 2015 | Diorio, C | 51 | 35 | 2 (6%) | 0 (0%) | NA | NA | NA | 2/35= 6% |
| 2014 | Ha, D | 27 | 23 | 0 (0%) | 0 (0%) | 12 | 1 (3%) | 0 (0%) | 1/27= 4% |
| 2013 | Rakha EA | 54 | 54 | 2 (4%) | 0 (0%) | NA | NA | NA | 2/54= 4% |
| 2012 | Sutton B | 38 | 22 | 0 (0%) | 0 (0%) | 16 | 5 (31%) | 0 (0%) | 5/38= 13% |
| 2011 | Jaffer S | 45 | 37 | 0 (0%) | 0 (0%) | 7 | 1 (14%) | 0 (0%) | 1/45= 2% |
| <2011 | Rakha EA* | 106 | 106 | 1 (1%) | 3 (3%) | 33 | 5 (15%) | 2 (6%) | 11/106= 10% |
| | | 652 | 516 | 11/516 (2.3 %) | 13/516 (2.5 %) | 144 | 14/144 (10%) | 11/144 (7.6%) | |
| | | | | **24/516 (4.6%)** | | | **25/144 (17.3%)** | | |

Figure 1 – 283

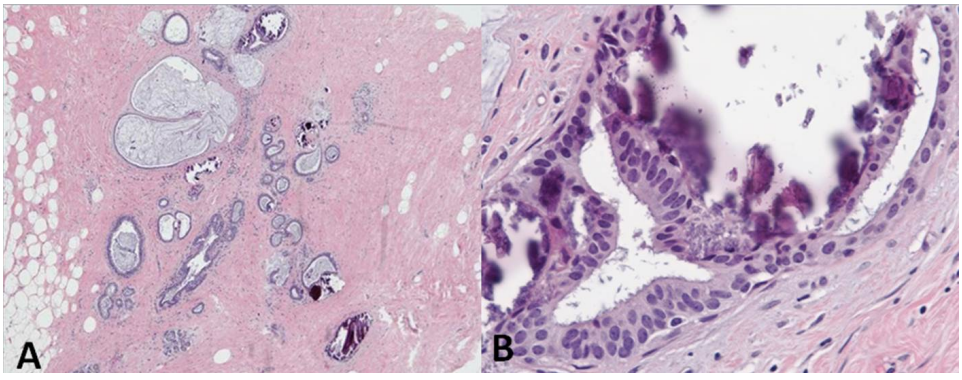
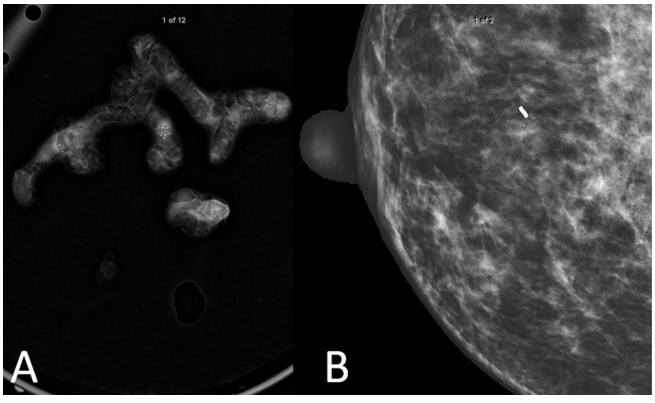



Figure 2 - 283




**Conclusions:** MLL has a very low risk of upgrade (<5%) when it presents without atypia on core needle biopsy and therefore consideration for imaging follow-up rather than surgical excision may be reasonable.

## 284 P16 Expression is correlated with Androgen Receptor (AR) expression in Triple-Negative Breast Cancers (TNBC)

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**Background:** Recent studies have shown that a significant proportion of basal-like and TNBCs have defects in $p16^{INK4a}$ signaling, which is a poor prognostic factor in breast carcinoma. AR is an emerging prognostic marker in TNBC and is expressed in 25-75% of TNBCs. In this retrospective study, we used a tissue microarray (TMA) to investigate p16 and AR expression in TNBC.

**Design:** TMAs were constructed using IHC-confirmed TNBCs and immunohistochemical stains for p16, CK5/6 and AR were performed. TMA and individual IHC stain slides were blindly scored by two pathologists. Nuclear and cytoplasmic staining of p16 in greater than 1% of cells was considered positive and the pattern was scored on a scale from 0-3 based on the extent of immunopositive cells (0, no staining; 1 < 25%; 2, 25-75%; 3, >75%). CK5/6 was scored positive (1) or negative (0) and AR expression greater than 1% was scored as positive. Statistical analysis was performed using spearman correlation to determine the relationship of IHC patterns between p16, CK5/6 and AR expression in TNBCs.

**Results:** A total of 75 of 126 cases had definitive tumor for all three biomarkers. Pearson's correlation for p16, CK5/6 and AR between two pathologists were 0.93, 0.92, 0.9, respectively. Forty-two cases had positive p16 expression (56.0%); 38 cases had p16 expression of 3+ (50.7%). There were 35 cases of CK5/6+ (46.7%) and 40 cases of CK5/6- (53.5%) TNBCs. The majority of cases in the TMA were AR- (n=51; 68%). There was no significant difference in p16 expression in CK5/6+ or CK5/6- TNBCs ($p > 0.1$). However, P16 expression was more frequently observed in AR- compared to AR+ TNBCs (42 cases (56.0%) vs 24 cases (32.0%)). Forty-two out of 51 AR- cases were P16+ (82.4%) and there was a negative correlation between p16 positivity and AR expression ($p < 0.005$, r=0.4).

| n = 75 (%) | P16 intensity in TBNCs | | | |
|---|---|---|---|---|
| 0 | 1 | 2 | 3 | Positive (1-3) |
| 12 (16.0) | 15 (20.0) | 10 (13.3) | 38 (50.7) | 42 (56) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| n=75 (%) | | P16 Intensity | | | | | Total |
| | | 0 | 1 | 2 | 3 | Positive (1-3) | n (%) |
| CK 5/6 | Pos | 2 (4.0) | 6 (8.0) | 5 (6.7) | 21 (28.0) | 32 (42.7) | 35 (46.7) |
| | Neg | 9 (12.0) | 9 (12.0) | 17 (22.7) | 5 (6.7) | 31 (41.3) | 40 (53.3) |
| | | | | | | | |
| AR | Pos | 3 (4.0) | 9 (12.0) | 3 (4.0) | 9 (12.0) | 21 (28) | 24 (32.0) |
| | Neg | 9 (12.0) | 6 (8.0) | 7 (9.3) | 29 (38.7) | 42 (56) | 51 (68.0) |

**Conclusions:** p16 expression is correlated with AR in TNBC independent of CK5/6 status ($p>0.1$) and may not infer characteristics of basal-like TNBC. Staining for p16 is may help identify more aggressive of TNBC with decrease expression of AR.

## 285 Implementation of the 2018 American Society of Clinical Oncology/College of American Pathologists Guidelines on HER2/neu Assessment by FISH in Breast Cancers: Predicted Impact in A Single Institutional Cohort

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**Background:** The 2018 ASCO/CAP Update modified the diagnostic criteria for determining human epidermal growth factor receptor 2 (HER2) status in several important ways, including the integration of immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) results, and/or the elimination of the "equivocal" category for specific subgroups (see table 1 for definition of HER2 subgroups). Additionally, employing reflex FISH testing with an alternative chromosome 17 probe (for HER2-equivocal cases with a dual

probe HER2/CEP17) is no longer recommended or necessary. The current study aimed to determine the predicted impact of implementing the 2018 ASCO/CAP guidelines on HER2 assessment by FISH in breast cancers, using data from a single institution.

**Design:** We reviewed consecutive cases of primary and metastatic breast carcinoma that had been assessed for HER2 status by FISH using dual probe HER2/CEP17 during a 48 month period. All had been scored using 2013 ASCO/CAP guidelines. HER2 status was then re-evaluated based on 2018 guidelines and the results were compared to the original interpretations. At our institution, HER2 status is routinely determined by both IHC and FISH on all breast cancers, and all FISH slides are manually counted by at least two technologists blinded to each other's results, each counting 20 tumor cell nuclei and calculating independent HER2/CEP17 ratios

**Results:** A total of 1542 cases were evaluated and assigned to different FISH groups as follows: Group 1: 189 (12.26%), group 2: 23 (1.5%), Group 3: 8 (0.5%), group 4: 144 (9.34%), and group 5: 1178 (76.4%), see table 1. By using the 2018 guidelines, 74 HER2 positive cases were re-classified as negative and 4 cases with equivocal results designated as HER2 positive. Overall the number of HER2 positive cancers were reduced by 70 cases (4.5% of all cases and 25.6% of HER2 positive cases), and those cases were emostly estrogen receptor positive (90%), progesterone receptor positive (80%), stage 1 (55%), and grade 1-2 (57.8%) cancers. Most (75.7%) of the newly HER2/neu negative cases had previously been classified as positive due to use of an alternative chromosome 17 probe on HER2-equivocal cases classified as such using 2013 ASCO/CAP criteria.

**Table 1: Classification of HER2 FISH groups by 2013 and 2018 guidelines**

| HER2 FISH Groups | Group definitions | FISH results by 2013 ASCO/CAP | | | FISH results by 2018 ASCO/CAP | |
|---|---|---|---|---|---|---|
| | | Positive | Negative | Equivocal | Positive | Negative |
| Group 1 (n=189) | HER2/CEP17 ? 2.0 and HER2 copy number ? 4.0 | 189 | 0 | 0 | 189 | 0 |
| Group 2 (n=23) | HER2/CEP17 ? 2.0 and HER2 copy number < 4.0 | 23 | 0 | 0 | 0 | 23 |
| Group 3 (n=8) | HER2/CEP17 < 2.0 and HER2 copy number ? 6.0 | 8 | 0 | 0 | 6 | 2 |
| Group 4 (n=144) | HER2/CEP17 < 2.0 and HER2 copy number ? 4.0 | 53 | 0 | 91 | 8 | 136 |
| Group 5 (n=1178) | HER2/CEP17 < 2.0 and HER2 copy number < 4.0 | 0 | 1178 | 0 | 0 | 1178 |
| Total (n=1542) | | 273 | 1178 | 91 | 203 | 1339 |

**Table 2: Comparison of FISH results between 2013 and 2018 guidelines**

| 2013 guidelines | 2018 guidelines | | Total |
|---|---|---|---|
| | Negative | Positive | |
| Negative | 1178 (76.4%) | 0 | 1178 (76.4%) |
| Equivocal | 87 (5.6%) | 4 (0.2%) | 91 (5.9%) |
| Positive | 74 (4.8%) | 199 (12.9%) | 273 (17.7%) |
| Total | 1339 (86.8%) | 203 (13.2%) | 1542 (100%) |

**Conclusions:** The net effect of implementing the revised 2018 HER2 guidelines is predicted to be a 25.6% reduction in HER2 positive results.

## 286 Does Monosomy of Chromosome 17 Define a Clinicopathologically-Distinct Subset of HER2/neu-Negative Breast Cancers?

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**Disclosures:** Somaye Zare: None; Juan Rong: None; Svenja Daehne: None; Farnaz Hasteh: None; Andres Roma: None; Marie Dell'Aquila: None; Oluwole Fadare: None

**Background:** Prior studies have shown that chromosome 17 aneusomy may influence the biology and clinical behavior of breast cancers, and the challenges that such alterations may introduce in the assessment of HER2/neu results from fluorescence in situ hybridization (FISH) are well known. Although monosomy of chromosome 17 in HER2/neu-amplified breast cancers is well studied, there is a notable

dearth of data on the significance of monosomy 17 in HER2/neu-negative cancers. Here, we aim to define the frequency of this finding in breast cancers, and to determine whether it defines a clinicopathologically-distinct subset of HER2/neu-negative cancer.

**Design:** The authors reviewed consecutive cases of primary breast carcinoma that have been diagnosed at a single institution during a 48-month period. All cases had been assessed for HER2/neu status using a dual probe HER2/CEP17 FISH assay and had been scored using 2013 ASCO/CAP guidelines. Monosomy 17 was determined to be present when HER2/neu and centromere enumeration probe (CEP 17) each displayed an average of <1.5 signals per cell. HER2/neu-non-amplified cases with monosomy 17 (Group A) were compared with other HER2 negative breast cancers with a HER2/neu and/or CEP 17 copy number ≥1.5 (Group B), regarding a variety of clinicopathologic features.

**Results:** A total of 1305 primary breast cancers were evaluated, 1060 of which were HER2/neu-negative. 40 (3.7%) of these 1060 HER2/neu-negative cases showed a HER2/neu and CEP 17 copy number of less than 1.5 (Group A), whereas the remaining 1020 HER2/neu-negative cases constituted Group B. Group A cancers were less frequently positive for estrogen receptor (65% versus 86.4%, p=0.000161) and progesterone receptor (57.5% versus 77.8%, p=0.002348) when compared with Group B patients. Additionally, Group A patients were significantly younger (Median ages: 51 versus 58 years, p<0.0001) and their tumors more frequently showed a "triple" negative immunophenotype (32.5% versus 11%). These two groups showed no significant differences regarding lymph node status, HER2/neu immunohistochemical scores, histologic grade, histotype, and stage distribution.

**Conclusions:** HER2/neu non-amplified breast cancers with monosomy of chromosome 17 account for 3.1% of breast cancers and may represent a clinicopathologically distinct subset of HER2/neu-negative cancers. These tumors were found to occur in significantly younger women and to display a "triple negative" phenotype more frequently than other HER2/neu-negative cancers

## 287 Triple-Positive Breast Carcinoma: A Histopathologic and Clinical Study of Patients Treated with Neoadjuvant Chemotherapy

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**Disclosures** Jennifer Zeng: None; Marcia Edelweiss: None; Dara Ross: None; Tracy-Ann Moo: None; Edi Brogi: None; Timothy D'Alfonso: None

**Background:** Approximately 50% of HER2+ breast carcinomas (BCs) also express hormone receptors (HRs), estrogen receptor (ER) and progesterone receptor (PR). HR+/HER2+ and HR-/HER2+ BCs differ in prognosis and response to systemic therapy. It is unclear whether ER+/PR+/HER2+ BCs, “triple-positive” (TPBCs), have unique morphology and clinical features. It has been suggested that TPBCs behave like luminal BCs and derive less benefit from HER2-targeted therapy, but information is limited. We studied the clinicopathologic features of TPBCs and assessed their response to neoadjuvant chemotherapy (NAC).

**Design:** We identified patients with TPBC post-NAC excised at our center between 2008-2018. HR and HER2 status were assessed by immunohistochemistry (IHC) +/- FISH in core biopsy samples. We studied the clinical and morphologic features of TPBCs. H-scores for HRs were calculated in core biopsies. Residual Cancer Burden (RCB) in post-NAC specimens was calculated.

**Results:** The TPBC study cohort consisted of 85 patients with a mean age 47 years (range 26-70). Mean pre-NAC tumor size was 3 cm (range 1.1-6.5). 46 (54.1%) patients were node-positive at diagnosis. There were 81 (95.3%) invasive ductal carcinomas (including 7 with mucinous and 5 with apocrine features) and 4 (4.7%) invasive lobular carcinomas. The median H-scores were 270 for ER and 150 for PR; 28 (32.9%) cases had high expression (>200) of both ER and PR. The median HER2/CEP17 FISH ratio was 2.8 and the median *HER2* signals/cell was 6.1.

AC-THP was the most common therapy regimen. 30 (35.3%) patients had pathologic complete response (pCR). 25 of 46 (54.3%) node-positive patients were downstaged to N0. Nottingham grade was significantly associated with pCR (p=0.006). pCR was significantly more frequent in HER2 IHC 3+ TPBCs than in IHC 0-2+ cases with FISH amplification (52% vs 9%, p<0.0001). RCB was significantly associated with distant recurrence (mean follow-up: 39.9 months) (p=0.01). The degree of ER and PR expression did not correlate with pCR, RCB, or distant recurrence.

| Characteristic<br><br>n=85 | n | % |
|---|---|---|
| **Mean age at diagnosis: years (range)** | 47 (26-70) | |
| **Histologic type** | | |
| Ductal | 81 | 95.3 |
| Lobular | 4 | 4.7 |
| **Nottingham grade** | | |
| 2 | 43 | 50.5 |
| 3 | 42 | 49.4 |
| **Pre-NAC clinical T classification** | | |
| cT1 | 14 | 16.5 |
| cT2 | 58 | 68.2 |
| cT3 | 9 | 10.6 |
| cT4 | 4 | 4.7 |
| **Pre-NAC nodal status** | | |
| Positive | 46 | 54.1 |
| Negative | 39 | 45.9 |
| **ER H Score** | | |
| 0-100 | 12 | 14.1 |
| 101-200 | 19 | 22.4 |
| 201-300 | 54 | 63.5 |
| **PR H Score** | | |
| 0-100 | 35 | 41.2 |
| 101-200 | 17 | 20.0 |
| 201-300 | 33 | 38.8 |
| **HER2 Immunohistochemistry** | | |
| 0/1+ | 5 | 5.9 |
| 2+ | 28 | 32.9 |
| 3+ | 52 | 61.1 |
| **HER2 FISH:**<br><br>Median HER2/CEP17 ratio (range)<br><br>Median HER2 signals/cell (range) | 2.8 (2 – 12.2)<br><br>6.1 (3.7-30.4) | |
| **RCB class** | | |
| 0 (pCR) | 30 | 35.3 |
| I | 13 | 15.3 |
| II | 25 | 29.4 |
| III | 17 | 20.0 |

**Conclusions:** TPBCs are morphologically heterogeneous and a few have mucinous, lobular, or apocrine features. Most TPBCs show high ER expression, and about a third have high expression of both ER and PR. The pCR rate of TPBCs is about 35%, much lower than that reported for HR-/HER2+ tumors. While HR expression did not correlate with pCR, HER2 IHC 3+ staining was associated with a better response to therapy and may help select patients for treatment in the neoadjuvant setting.

## 288 Frequency, Clinicopathologic Characteristics and Follow-up Outcomes of Non-Pleomorphic Invasive Lobular Carcinoma of Breast with HER-2 Overexpression: A Retrospective Analysis of 10-Year Study in an Academic Institution

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**Disclosures:** Huina Zhang: None; Rana Ajabnoor: None; Ioana Moisini: None; Bradley Turner: None; Xi Wang: None; David Hicks: None

**Background:** The non-pleomorphic invasive lobular carcinoma (ILC) has been traditionally considered to be HER-2 negative. Only one study which described twelve cases of classical-type ILCs with HER-2 overexpression and rare case reports have been reported. There is a dearth of studies on this topic, especially assessing the clinicopathologic characteristics and long-term follow-up outcomes.

**Design:** Non-pleomorphic ILCs with HER-2 overexpression were retrospectively identified during 2008-2018. The clinicopathologic characteristics (age, tumor size and stage, lymph node status), histologic features (morphologic type and nuclear grade), prognostic variables (estrogen receptor (ER), progesterone receptor (PR), HER-2 and Ki-67) and clinical follow-up information (administration of HER-2 targeted therapy and outcome) were collected.

**Results:** A total of 16 patients were identified, accounting for ~3.5% of ILCs. Among them, four were detected by immunohistochemistry and 12 by Fluorescence In Situ Hybridization (FISH). The mean age was 71 years old and the average tumor size was 3.4 cm. 62.5% (10/16) were ER+/PR+, 18.75% were ER+/PR- and 18.75% were ER-/PR-. For FISH-identified cases, the average ratio of HER-2/CEP17 was 4.8 and the average HER-2 copy number was 7.9. Morphologically, Ten cases were classic type and 6 were variant types including histiocytoid (1), solid (3), alveolar (1) and trabecular (1). Thirteen cases had grade 2 nuclei and 3 had grade 1 nuclei. The average Ki-67 index was ~22%. Three cases had positive lymph nodes including 2 micrometastasis only. Fourteen patients received HER-2-based chemotherapy either in neoadjuvant (3) or adjuvant (11) setting. None of the patients who received neoadjuvant therapy achieved pathologic complete response. Among the patients without neoadjuvant treatment, the pathologic stages were pT1c (1), pT2 (8), and pT3 (2). One patient had stage IV disease at the diagnosis and was given neoadjuvant chemotherapy with a final stage of ypT1aypN0. However, she died after 17 months of follow-up. All the other patients were alive without evidence of disease with an average of 35 months of follow-up.

**Conclusions:** This is by far the largest investigation focusing on the non-pleomorphic ILCs with HER-2+ overexpression. Variant histology, loss of hormonal receptors, especially negative PR with grade 2 nuclei may be associated with HER-2 overexpression in non-pleomorphic ILCs. The majority of patients received HER-2 based therapy and had a relatively good prognosis.

## 289 The Predictive and Prognostic Value of FOXP3+ and FOXP3+/CD25+ Regulatory T Cells, and PD-L1 Expression in Triple Negative Breast Cancer

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**Disclosures:** Lin Zhang: None; Lei Wang: None; Xiaohong Iris Wang: None; Jianmin Ding: None; Qigang Sun: None; Songlin Zhang: None

**Background:** Tumor microenvironment has been increasingly recognized as a major regulator of the carcinogenesis. Tumor-infiltrating lymphocytes (TILs) can be used to monitor host immune response and has been shown to be both predictive and prognostic in triple negative breast cancer (TNBC). In this study, we evaluated the predictive and prognostic value of FOXP3+ TILs, FOXP3+/CD25+ TILs, and PD-L1 expression in TNBC.

**Design:** Tissue microarrays of biopsy and resection specimens from 58 TNBC patients who underwent neoadjuvant therapy (NACT) were prepared. The number of FOXP3+ and FOXP3+/CD25+ TILs, and PD-L1 expression in both tumor cells and TILs were assessed by immunohistochemistry. ImageJ digital analysis was used for quantification of FOXP3+ and FOXP3+/CD25+ TILs. PD-L1 expression and score was manually evaluated and calculated by a breast pathologist (SZ). The continuous variables were analyzed using student's t-test, whereas the categorical data were evaluated using chi-square test. Overall survival was plotted on Kaplan-Meier survival curve.

**Results:** Among the 58 TNBC patients, 45% (26) of patients achieved pCR following NACT whereas 55% (32) of patients still had residual disease. The median overall survival was 41 months (range, 15-123 months). FOXP3+ and FOXP3+/CD25+ lymphocytes had strong positive correlation (R=0.89), and they also showed strong association with PD-L1+ TILs (p=0.03) and PD-L1 combined positive score (CPS) (p=0.0001). NACT significantly reduced FOXP3+ and FOXP3+/CD25+ TILs when compared the original biopsy with the resection specimens. PD-L1+ tumor cell score, PD-L1+ TILs score, and PD-L1 CPS correlated with pCR (p=0.04, 0.01, and 0.01, respectively), but FOXP3+ and FOXP3+/CD25+ lymphocytes were not associated with pCR. For Kaplan-Meier survival analysis, high FOXP3+/CD25+ lymphocytes (Figure 1A) and PD-L1 CPS (Figure 1B) were associated with better overall survival.

Figure 1 - 289

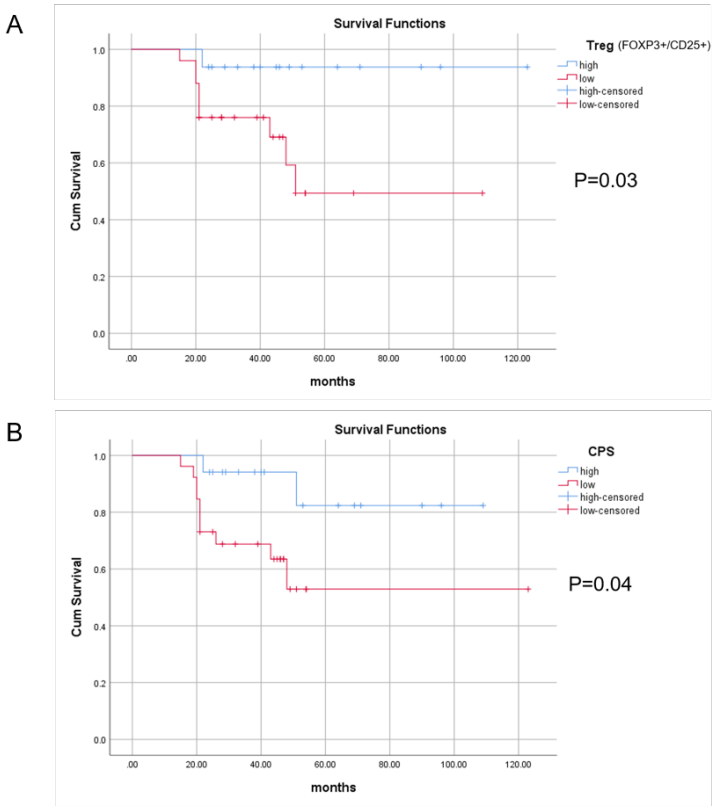


Figure 1: A. Increased FOXP3+/CD25+ Tregs are associated with better overall survival. B. High PD-L1 combined positive score is associated with better overall survival.

**Conclusions:** Our results shows although FOXP3+ and FOXP3+/CD25+ lymphocytes have a strong positive correlation (R=0.89), they are not identical. FOXP3+/CD25+ lymphocytes may be a better marker for evaluating regulatory T (Treg) cells than FOXP3 alone. Evaluation of PD-L1 score and FOXP3/CD25+ lymphocytes in TNBC has both therapeutic and prognostic value.

## 290 Predictive Value of HER2 Fluorescence In Situ Hybridization (FISH) Result in HER2 Positive Breast Cancer Response to Neoadjuvant Therapy

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**Disclosures:** Jing Zhao: None; Aili Suo: None; Chao Zhang: None; Qiuying Shi: None; Zhimin Wei: None; Zaibo Li: None; Xiaoxian Li: None

**Background:** Dual probe fluorescence in situ hybridization (FISH) analysis is routinely used to evaluate HER2 gene amplification in breast cancer. Both HER2/CEP 17 ratio (HER2 ratio) and HER2 average copy number per cell (HER2 copy #) are routinely evaluated. Although HER2 positive (HER2+) breast cancers generally respond well to HER2 targeted therapy, a significant number of cases do not respond and the HER2 targeted therapy is not without side effect. This study aimed to find a cutoff value of HER2/CEP 17 ratio and HER2 copy# to predict HER2+ breast cancer response to therapy.

**Design:** *Seventy HER2 FISH positive (according to 2018 ASCO/CAP recommendation) breast cancer biopsy cases from 2010 to 2016 were retrieved. All patients were treated with neoadjuvant HER2 targeted therapy and chemotherapy. Tumor response was evaluated on the excisional specimens. Cancers with pathologic complete response (pCR) or MD Anderson residual cancer burden-I (RCB-I) were classified as responder and cancers with RCB-II and III as non-responder. The prediction power of the two biomarkers was analyzed by the receiver operating characteristics (ROC) model.*

**Results:** HER2 ratio of 8.70 had 90% sensitivity for responders and HER2 copy# of 21.6 had 89% sensitivity for responders. HER2 ratio of 2.20 had 95% specificity for non-responders and HER2 copy# of 5.1 had 95% specificity for non-responders. Combining HER2 ratio and copy# was not superior than HER ratio and copy# separately to predict tumor responding rate (p=0.531, 0.416 respectively). The areas under ROC curve (AUC) were 0.759, 0.766, and 0.785 for HER2 ratio, HER2 copy #, and combining the two biomarkers, respectively.

**Conclusions:** HER2+ breast cancer with a ratio >8.70 or HER2 copy# >21.6 is highly likely to respond to HER2 targeted therapy. HER2+ cancer with a ratio <2.20 or HER2 copy# <5.1 is unlikely respond to HER2-targeted therapy.

## 291 HER2 Immunohistochemistry Positivity is Strongly Predictive of HER2 Positive Breast Cancer Response to Neoadjuvant Therapy

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**Disclosures:** Jing Zhao: None; Chao Zhang: None; Aili Suo: None; Qiuying Shi: None; Zhimin Wei: None; Zaibo Li: None; Uma Krishnamurti: None; Xiaoxian Li: None

**Background:** The current recommendation is to reflect HER2 immunohistochemistry (IHC) equivocal breast cancer cases to fluorescence in situ hybridization (FISH) analysis. Either IHC 3+ or FISH positive cancers are considered HER2 positive (HER2+) and treated with HER2 targeted therapy. The predictive value of IHC or FISH positivity in tumor response to HER2 targeted therapy is not very clear.

**Design:** Ninety-one HER2+ breast cancer biopsy cases from 2010 to 2016 were retrieved. All patients were treated with neoadjuvant HER2 targeted therapy and chemotherapy. Tumor response was evaluated on the excisional specimens. Cancers with pathologic complete response (pCR) or MD Anderson residual cancer burden-I (RCB-I) were classified as responder and cancers with RCB-II and III as non-responder. The cases were grouped as IHC3+/without FISH (n=26), IHC3+/FISH+ (n=41), and IHC1-2+/FISH+ (n=24) and correlated with tumor response. The 2018 ASCO/CAP recommendations were followed. Other clinicopathologic parameters including patient age, tumor size, nuclear grade, mitosis, Nottingham grade, ER, PR and Ki-67 were also evaluated and correlated with response.

**Results:** In the IHC3+/FISH+ group, 30 of 41 (73.2%) were responders; 18 of 26 (69.2%) IHC3+/without FISH were responders and 7 of 24 (29.2%) IHC1-2+/FISH+ were responders. IHC1-2+/FISH+ was significantly associated with non-responder in univariate analysis and multivariate analysis after adjusting for other clinicopathologic parameters. In addition, IHC3+ was significantly associated with high FISH HER2 ratio and copy number.

**Conclusions:** HER2 IHC3+ is strongly associated with HER2+ breast cancer response to neoadjuvant therapy. IHC evaluation remains critical in breast cancer biomarker evaluation.

## 292 Predictive Biomarker of Response to Neoadjuvant Therapy in HER2 Positive Breast Cancer

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**Disclosures:** Jing Zhao: None; Jane Meisel: None; Aili Suo: None; Chao Zhang: None; Zhimin Wei: None; Ritu Aneja: None; Zaibo Li: None; Rita Nahta: None; Xiaoxian Li: None

**Background:** HER2 targeted neoadjuvant therapy has high efficacy in treating HER2 positive (HER2+) breast cancer. This study examined clinicopathologic features in predicting response to neoadjuvant therapy in HER2+ breast cancer.

**Design:** Totally 98 HER2+ breast cancer biopsies from 2010 to 2016 were retrieved. All patients were treated with neoadjuvant therapy including HER2 targeted therapy (transtuzumab or pertuzumab or both) and chemotherapy agents. Tumor response was evaluated on excisional specimens using the MD Anderson residual cancer burden (RCB) classification. Tumors with pathologic complete response (pCR, no residual invasive carcinoma in the breast and lymph nodes) and RCB-I were classified as responder and tumors with RCB-II and III as non-responder. Patient age, tumor size, nuclear grade (1/2 vs 3), mitosis, Nottingham grade, HER2 IHC (1/2+ vs 3+), HER2/CEP17 ratio, HER2 copy number, ER, PR, Ki-67 and tumor infiltrating lymphocyte (TIL) were evaluated and correlated with response. TIL was evaluated as average and hot spot/total tumor stromal ratio.

**Results:** Young age, small tumor size, nuclear grade 1/2, HER2 IHC 3+, high Ki-67, high HER2/CEP17 ratio, and high HER2 copy number were significantly associated with good response (responder) in univariate and multivariate analysis (all P<0.05). TIL was not associated with response after adjusting for other covariates in multivariate analysis.

**Conclusions:** Clinicopathologic features may help to predict HER2+ breast cancer response to neoadjuvant therapy. TIL is not predictive of HER2+ breast cancer response to neoadjuvant therapy in this cohort.

## 293 Is Excision Required for All Patients with Core Needle Biopsy Diagnosis of Intraductal Papilloma?- A Retrospective Analysis of a High-Risk Population in a Safety-Net Hospital

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**Disclosures:** Xiaofeng Zhao: None; Kathleen Reilly: None; Suzanne Pascarella: None; Hang Zhou: None; Congli Wang: None; Suad Taraif: None

**Background:** The management of benign intraductal papilloma (IDP) diagnosed on core needle biopsy (CNB) remains controversial. This study was conducted to evaluate the rate of upgrade to atypical ductal hyperplasia (ADH) or malignancy following excision, identify factors associated with such upgrade and the influence of upgrade rate on further management options.

**Design:** Retrospective review of institutional pathology archives from January 2006 to August 2018 revealed 195 IDP by CNB, of which 147 lesions (from 142 female patients) subsequently underwent surgical excision. Age range was 30 to 83 years (median 55 years). The excisional biopsy diagnoses were compared with CNB results.

**Results:** The majority of excised papillomas were diagnosed as IDP on CNB (139, 95%); only 8 cases had a CNB diagnosis of atypical intraductal papilloma (AIDP; 5%). At excision, out of 139 cases initially diagnosed as IDP with no atypia on CNB, 18 (12.95%) were found to have ADH, 14 (10.07%) were upgraded to ductal carcinoma *in situ* (DCIS) and 2 were upgraded to invasive carcinoma (1.44%). The overall rate of upgrade of IDP to ADH or malignancy in surgical specimens is 24.46%. 2 of 8 AIDP (25.0%) were upgraded to DCIS after surgical excision. The presence of both ADH and usual ductal hyperplasia (UDH) elsewhere in the biopsy carries a higher rate of upgrading to malignancy (40.0%) than ADH or UDH alone (12.5% and 10%, respectively). Of the 147 IDP cases, 66 were radiologically identified to be located in a central duct and 75 were in peripheral ducts. Location of IDP had no association with upgrade of lesion. Review of the pre-biopsy imaging findings identified no specific features to predict lesion upgrade at surgical excision.

**Conclusions:** In our patient population, the rate of upgrade to a higher risk lesion at excision of IDPs with or without atypia is similar (25.00% and 24.46% respectively). The presence of both ADH and UDH on CNB in addition to IDP significantly increase the risk of having a malignancy at excision. Radiologic features are not specifically helpful in predicting the upgrade. Surgical excision should be considered for all IDPs.

## 294 Performance of Frozen Section Evaluation on Sentinel Lymph Nodes in Breast Carcinoma: A Retrospective Study in a Safety-Net Hospital

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**Disclosures:** Xiaofeng Zhao: None; Congli Wang: None; Suad Taraif: None

**Background:** Sentinel lymph node (SLN) biopsy was introduced to be an alternative to axillary lymph node dissection (ALND) in early 1990s. Axillary lymph node status is among the most important prognostic factors in breast carcinoma. Intraoperative assessment of SLN by frozen section allows an immediate ALND and avoiding reoperation. However, metastasis can be missed during the frozen section of sentinel lymph nodes, making the routine use of frozen section for the evaluation of SLN biopsy questionable.

**Design:** We performed a ten-year retrospective review of the performance of SLN using frozen section to evaluate its sensitivity, specificity, positive and negative-predictive values.

**Results:** Between January 2008 and August 2018, a total of 464 SLNs from 206 patients (24-85 year old; median age 57) underwent intraoperative evaluation by frozen section. The median number of SLNs dissected per patient was 2 (range 1-6). A total of 97 lymph nodes were confirmed to be positive on permanent section, with 63 diagnosed as “positive”, 7 as “suspicious” and 4 as “atypical” on frozen section. There were 23 positive lymph nodes missed on frozen section. Among them, 5 were with tumor cells only present on permanent sections, and tumor cells only identifiable by IHC in 9, leaving only 9 truly false negative cases. Most lymph nodes with “atypical” frozen diagnosis turned out to be negative (10 out of 14), and majority of “suspicious” lymph nodes on frozen were confirmed to be positive (7 out of 9). For analysis purposes, atypical was considered to be negative and suspicious to be positive. The overall performance of frozen section on sentinel lymph nodes is displayed in Table 1.

| Frozen evaluation | Final diagnosis | | Total |
|---|---|---|---|
| | positive | negative | |
| Positive | 63 | 0 | 63 |
| Suspicious | 7 | 2 | 9 |
| Atypical | 4 | 10 | 14 |
| Negative | 9 | 369 | 378 |
| Total | 83 | 381 | 464 |
| Sensitivity | | 84.3% | |
| Specificity | | 99.5% | |
| Positive predictive rate | | 97.2% | |
| Negative predictive rate | | 96.7% | |
| False negative | | 10.8% | |

**Conclusions:** In this study, we determined that intraoperative evaluation of sentinel lymph node by frozen section in our facility shows high sensitivity, specificity, as well as positive and negative predictive rate. The discordance between frozen section analysis and definitive diagnosis of SLN biopsy occurred in 10.8%in patients with breast cancer, comparable to other studies. Intraoperative evaluation of sentinel lymph node by frozen section still provides valuable information to avoid unnecessary ALND.

## 295 Papillary Lesions in Male Breasts: A Study of 113 Cases Demonstrates a Broad Clinicopathological Spectrum

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**Disclosures:** Elaine Zhong: None; Esther Cheng: None; Michael Goldfischer: None; Syed Hoda: None

**Background:** Papillary (pap) lesions in male breasts (PLMB; including those that are benign, atypical, and malignant: non-invasive [inv] and inv carcinoma [ca]) are uncommon. To date, PLMB have been reported as individual cases and relatively small series.

**Design:** The departmental (including consultation) database was searched for PLMB over an 18-year period (2000-2018). All available material, including clinical and histopathological, was reviewed and assessed. WHO 2013 nomenclature and criteria for pap ca were used. ER, HER2, Ki67, smooth muscle myosin (SMM), p63, and CK5 immunostains were reviewed, whenever available.

**Results:** 113 PLMB, in 112 patients, were identified (**Table 1**). At presentation, the youngest patient (with neurofibromatosis 1 and diffuse papillary hyperplasia) was 7-month-old (**Fig. 1**), and the oldest was 88-year-old. The most common presentations were mass (55%, 11/20) and nipple discharge (30%, 6/20). The largest pap mass (encapsulated pap ca) spanned 4.4 cm. Two remarkable cases were encountered: unilateral adenomyoepithelioma (cystic, without atypia, in a 73-year-old) and a unilateral solid-pap DCIS (in a 57-year-old with Klippel-Feil syndrome, 6 months after diagnosis of a contralateral inv pap breast ca with metastasis from latter). No case of florid papillomatosis or of metastatic pap ca was identified. On CK5, papillary hyperplasia generally showed 3 layers, similar to gynecomastia (Am J Surg Pathol 2012;36:762-768). CK5 stained myoepithelium with higher intensity and in greater proportion than SMM. 3 cases of non-inv pap ca showed hybrid (>2 types) morphology, with 1 dominant type. In solid-pap ca, 16% (6/38) had mucinous features (**Fig. 2**). In pap ca, nuclear grade was 1 or 1-2 in 19% (10/53), 2 in 68% (36/53), and 2-3 or 3 in 13% (7/53). Microinvasive (<1 mm) ca was associated with 20% (20/102) of non-inv pap ca cases. All pap ca were ER(+) and HER2(-). The most common surgical procedure was excision (57%); 11 patients had mastectomy. Axillary nodal involvement was present in 1/7 of inv pap ca and 1/20 of microinvasive ca. In cases with follow-up, no inv pap ca showed distant metastasis or proved fatal, and no non-inv pap ca recurred.

| | **n** | **Age, mean** | **Age, median** | **Age, range** |
|---|---|---|---|---|
| Pap hyperplasia | 5 | 36.3 | 47 | 7 months-49 |
| Intraductal Papilloma* | 1 | 60.0 | 60 | 60-60 |
| Atypical Papilloma | 7 | 63.8 | 68 | 37-81 |
| Pap DCIS** | 48 | 61.3 | 60.5 | 19-88 |
| Encapsulated Pap Ca** | 15 | 68.7 | 68 | 55-82 |
| Solid Pap Ca** | 38 | 64.4 | 65 | 41-88 |
| Inv Pap Ca | 7 | 68.7 | 68 | 49-88 |

*not including 1 case of adenomyoepithelioma
**including microinvasive ca cases
Cases that had invasive *and* noninvasive carcinoma are included in both categories.

Figure 1 - 295

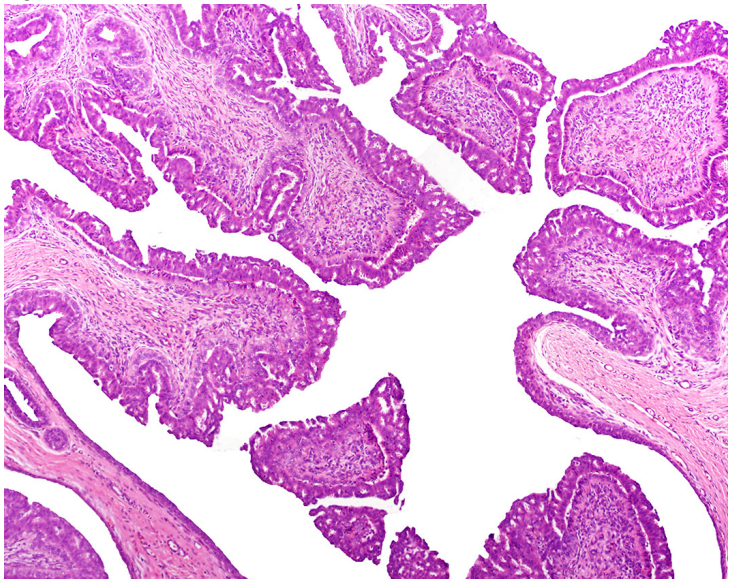
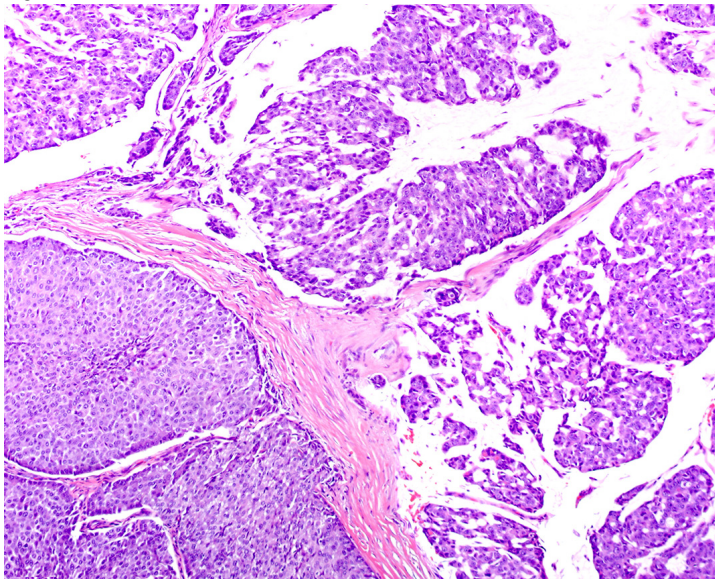


Figure 2 - 295



**Conclusions:** The spectrum of PLMB is broad—and ranges from pap hyperplasia to inv pap ca. Pap DCIS is the most common PLMB. Inv pap ca is uncommon and is ER(+) and HER2(-). WHO classification of pap ca can be applied in males, however some non-inv pap ca display hybrid morphology.

## 296 Intense Basolateral Membrane Staining Indicates HER2 Positivity in Invasive Micropapillary Breast Carcinoma

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**Disclosures:** Shuling Zhou: None; Qianming Bai: None; Wentao Yang: None

**Background:** According to the 2018 American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP) human epidermal growth factor receptor (HER2) testing recommendation, a positive HER2 (3+) test requires that >10% of invasive tumor cells exhibit intense and complete membrane staining. Invasive micropapillary carcinoma (IMPC) is characterized by the inside-out growth of tumor clusters in a pseudopapillary arrangement. Based on the 2018 ASCO/CAP guideline, IMPC tumors with moderate to intense but incomplete staining should be reported as 2+, requiring an additional test. And the criteria of HER2 3+ for IMPC are not mentioned in the guideline.

**Design:** One hundred and forty seven cases with varied proportions (10%-100%) of IMPC with moderate to intense incomplete HER2 membrane staining between 2010 and 2017 were retrieved and reviewed. Immunohistochemical characteristics including estrogen receptor (ER), progesterone receptor (PR), HER2, Ki67 and epithelial membrane antigen (EMA) were reviewed. Fluorescence in situ hybridization (FISH) was performed on all the cases.

**Results:** All IMPC components exhibited reversed/peripheral polarity. The majority of cases were of the luminal subtype (122/147, luminal A, 36/147, 24.5%; luminal B, 86/147, 58.5%), and the HER2 subtype and triple negative subtype accounted for 15.6% (23/147) and 1.4% (2/147), respectively. IMPC components of all 147 tumors exhibited incomplete basolateral HER2 membrane staining in more than 10% of tumor cells. All of them were scored as HER2 2+ based on the 2018 ASCO/CAP recommendation. One hundred and sixteen of the tumors (116/147, 78.9%) had moderate staining, and 36 (36/116, 31%) showed HER2 amplification by FISH, and the HER2 gene was amplified in all the remaining 31 tumors (31/147, 21.1%) that exhibited intense basolateral membrane staining. Altogether, HER2 gene amplification was identified in 67 (67/147, 45.6%) cases, including all tumors with intense membrane staining and 36 with moderate staining.

**Conclusions:** Our study showed that IMPC with intense basolateral membrane immunostaining indicates HER2 positivity, even if the staining is incomplete, it should be classified as 3+ rather than 2+, which will avoid the patients missed the opportunity of targeted therapy due to lack of conditions for FISH detection and shorten the detection time of clearing the HER2 gene status. The ultimate aim is to ensure that all patients with HER2 gene amplification receive targeted therapy.

## 297 Measuring the Association of Proliferative Breast Lesions to Carcinoma

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**Disclosures:** Yonah Ziemba: None; Feifan Chen: None; Shaul Gold: None; Mallorie Angert: None; Kalpana Reddy: None; Morris Edelman: None

**Background:** With the advancements of imaging techniques, there is increased frequency of radiologically worrisome lesions that turn out to be benign. Proliferative disease without atypia (PDWA) is a class of benign lesions that includes adenosis, usual ductal hyperplasia (UDH), radial scar, flat epithelial atypia (FEA) and papilloma. It is well-accepted that adenosis and UDH are not associated with significant increase in cancer risk and do not require excision. However, management of radial scar, FEA and papilloma is a matter of debate. In seeking to quantify the association of these lesions with cancer, we examined the frequency in which these lesions are found in the same biopsy specimen as invasive cancer.

**Design:** Pathology department records were retrospectively searched for all breast core biopsies that were diagnosed from January 2016 through June 2018. Where an accession number included more than one site, each location was evaluated separately, totaling 14,387 biopsy sites. For each lesion of interest, we calculated the total number of all cases of the lesion, and also the number of cases in which the lesion was found together with invasive cancer. The primary outcome was expressed as a percent to represent the rate at which the lesion was associated with cancer. While our primary interest was radial scar, FEA and papilloma, we also evaluated adenosis and UDH as negative controls and DCIS and ADH as positive controls.

**Results:** Invasive carcinoma was found in: 802 out of 1,325 biopsies with DCIS (61%); 79 out of 404 biopsies with ADH (20%); 6 out of 68 cases of FEA (9%); 19 out of 2423 cases of adenosis (8%); 4 out of 174 cases of radial scar (2%); 9 out of 774 cases of papilloma (1%), and 11 out of 1692 biopsies with UDH (<1%).

**Conclusions:** This dataset show a high association of invasive cancer with CIS & ADH, a moderate association with FEA & adenosis and a very low associations with radial scar & papilloma. The veracity of the FEA association is less reliable given the small population of FEA cases in our dataset. This analysis is based on original pathology reporting, not on slide review, and therefore the influence of reporting bias cannot be ascertained. The lower association of reported invasive radial scar and papilloma compared to the controls is consistent with the newer studies that show that these lesions have very low risk.

## 298 Intensity of Ki67 Staining as an Adjuvant Predictor of Response to Neoadjuvant Therapy in Patients with High Ki67

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**Disclosures:** Patricija Zot: None; Lorraine Colon Cartagena: None; Raghavendra Pillappa: None; Michael Idowu: None; Valentina Robila: None

**Background:** Proliferation index Ki67 is being increasingly used in the setting of neoadjuvant chemotherapy (NAC) as a predictor of both pathologic response and post-treatment prognosis. Only the percentage of Ki67 positive cells is currently being reported. However, preliminary mouse model studies show that the intensity of Ki67 in addition to percentage positivity may also play a role in response to treatment. The purpose of this study was to evaluate the correlation of the intensity of percent positive Ki67 tumor cells to the pathological response to neoadjuvant treatment.

**Design:** Needle core biopsies with similar fixation protocols and immunohistochemical stains for Ki67 (MM1 and MIB-1 clones) of patients treated with NAC (2010- 2016) were evaluated independently by two breast pathologists. The percentage of positive cells was recorded. The cut-off of 20% was used to differentiate between percent low and high positive tumor cells. We also evaluated the intensity of staining and defined low intensity cases as predominantly 1-2+ (mild to moderate) and high intensity cases as predominantly 3+ (strong) staining (Fig. 1). The Ki67 expression profiles were then correlated with the tumor hormonal status and response to NAC. Chi square statistical analysis was performed.

**Results:** 152 patients who underwent NAC treatment had the Ki67 staining of the needle core biopsies retrospectively reviewed. Using a 20% cutoff, a high Ki67 proliferation index significantly favors a complete versus partial or no response (p=0.004) (Table 1). At high Ki67, cases with high intensity are also more often associated with complete vs probable response. Triple negative carcinomas have a statistically significant increased prevalence of high percent/ low intensity Ki67 compared with Her2 positive or luminal phenotypes (p=0.02) (Fig. 2). However, this distribution does not differentially impact the rates of complete or partial response to NAC. Instead, the only cases with high percent/ low intensity Ki67 and no definite response are triple negative.

| Response to NAC | Low Ki67 | | High Ki67 | |
|---|---|---|---|---|
| | Low intensity | High Intensity | Low intensity | High Intensity |
| **Complete response (n=38)** | 1 (2.50%) | 1 (2.5%) | 8 (20.00%) | **30 (75%)** |
| **Probable response (n=89)** | 6 (6.74%) | **18 (20.22%)** | 15 (16.85%) | **50 (56.18%)** |
| **No definite response (n=23)** | 1 (4.34%) | 9 (39.13%) | 4 (17.39%) | 9 (39.13%) |

Table 1. Response to neoadjuvant therapy stratified based on Ki67 percentage and intensity

Figure 1 – 298

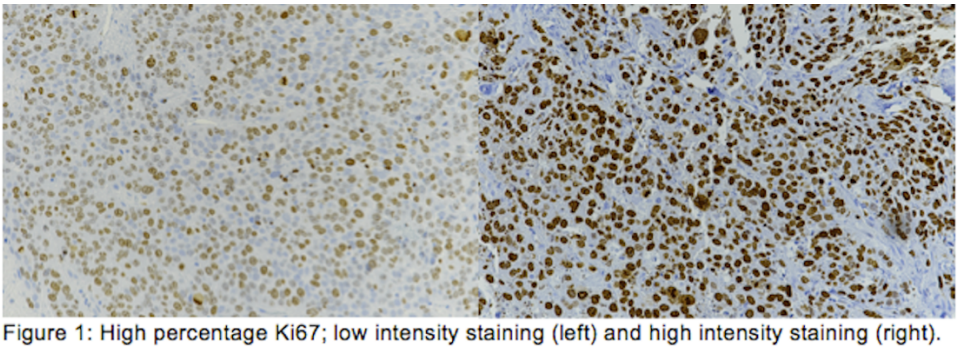

Figure 1: High percentage Ki67; low intensity staining (left) and high intensity staining (right).

Figure 2 - 298

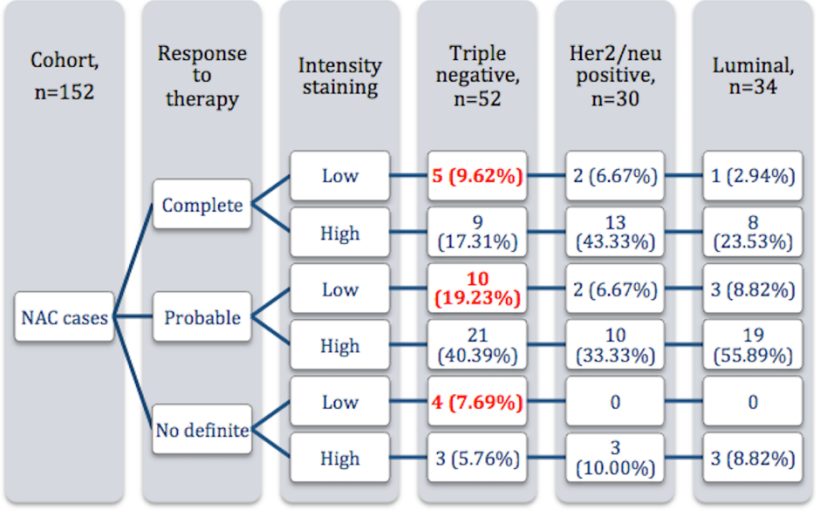


Figure 2: Response to therapy by phenotype in the High Ki-67 group.

**Conclusions:** In our cohort, a cutoff of 20% for Ki67 proliferation index significantly correlates with complete pathological response to treatment. A higher proportion of cases with Ki67 high percent/ low intensity are seen in triple negative carcinomas. While this profile does not correlate with a distinctive rate of complete or partial response, it is enriched in patients that do not achieve a measurable response to NAC.