The histological diagnosis of LGOS is often challenging. Because of the bland cytology and mature bony trabeculae, LGOS tends to be confused with benign lesions. POS, for example, may be mistaken as myositis ossificans (MO), osteochondroma (OC), and other surface lesions, while LGCOS may simulate fibrous dysplasia (FD) among others. The accurate diagnosis is mandated to allow appropriate management. Since benign mimics of LGOS are not expected to harbor the characteristic gene amplification of sarcoma, we reasoned MDM2 and CDK4 immunostains may aid in this difficult differential diagnosis.

Design: Twenty-two cases of LGOS from 20 patients (14 POSs, 7 LGCOSs, 1 LGOS of undetermined subtype), and 38 cases of benign histological mimics of LGOS (11 MOs, 14 FDs, 6 OCs, 1 desmoplastic fibroma, 4 florid reactive periostitides, 1 Nora's lesion, and 1 Turrett exostosis) were retrieved from the hospital files. A representative section of each case was immunostained with antibodies against MDM2 and CDK4. The results were expressed with intensity graded from 1 (weak) to 3 (strong), and with extent being either focal (1-10%) or diffuse (11-100%).

Results: Fourteen LGOSs labeled for MDM2 (14/22, 64%); and 19 cases (19/22, 86%) labeled for CDK4. All the LGOSs (22/22, 100%) expressed one or both of the markers, with 11 cases expressing both (11/22, 50%). In the majority of the cases, staining was diffuse (20/22, 91%) and in moderate or strong intensity (15/22, 68%) for either antibody. In contrast, none (0%) of 38 benign lesions demonstrated immunoreactivity for MDM2 or CDK4. The combination of these two markers thus made both sensitivity and specificity reach 100% for the diagnosis of LGOS.

Conclusions: All the LGOSs labeled for MDM2 and/or CDK4, while none of benign histological mimics expressed these markers in this analysis. MDM2 and CDK4 immunostaining may hence serve as a useful adjunct for the diagnosis of POS and LGCOS.

125 Lipomatous Neoplasms: The Diagnostic and Prognostic Implications of Molecular Classification

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Background: The use of molecular techniques has been advocated in differentiating lipomas from atypical lipomatous tumors/well-differentiated liposarcomas (ALT/WDL). However, considering that both groups of tumors can recur locally but lack metastatic potential, the practical implication of their molecular characterization has not been scrutinized. The aim of this study was to assess the clinical value of the molecular classification of lipomatous neoplasms.

Design: Four hundred five cases of lipomatous neoplasms diagnosed between 1990 and 2000 and located in the extremities were analyzed for the presence of *MDM2/CPM* amplification using fluorescence in situ hybridization (FISH). One hundred cells were analyzed in each tumor. Local recurrence-free survival was estimated with the Kaplan-Meier method and compared with the log rank test. Multivariate analysis was conducted using the Cox regression model.

Results: Using solely histologic assessment as criteria, the 405 tumors were classified as lipoma (n=324), intramuscular lipoma (n=29) and ALT/WDL (n=52). After molecular analysis, 11 of the tumors histologically classified as ALT/WDL were reclassified as lipoma (n=5) and intramuscular lipoma (n=6), whereas 7 of the tumors histologically designated as lipoma were reclassified as ALT/WDL. Follow-up information was available for 303 tumors. Prior to the molecular data, the 5-year local recurrence rates for lipoma, intramuscular lipoma and ALT/WDL were 2%, 5% and 45%, respectively (P <0.0001). After molecular reclassification, the 5-year local recurrence rates for lipoma, intramuscular lipoma and ALT/WDL were 1%, 12% and 44%, respectively (P <0.0001). Multivariate analyses showed that histologic type emerged as the only independent risk factor before molecular classification (HR_{alt}=4.15; 95% CI, 1.70-11.13; p=0.0005); however, after molecular classification both histologic subtype (HR_{alt}=2.62; 95% CI, 1.28-5.58, p<0.0001) and type of surgery (HR_{wle}=0.59; 95% CI, 0.36-0.97; p=0.036) correlated with the risk of local recurrence.

Conclusions: The use of molecular testing to complement the histologic assessment of lipomatous tumors more precisely discriminates local recurrence risks for individual groups of lipomatous tumors located in the extremities and therefore provides for appropriate surgical management decisons.

126 MicroRNA (miRNA) Microarray Analysis in Well-Differentiated (WD) and Dedifferentiated (DD) Liposarcomas (LP)

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Background: The function of a microRNA is to silence multiple target genes. Depending upon its target genes, miRNA can function to promote or suppress cell growth. Abnormal miRNA expression has been shown in various tumors including liposarcomas. We hypothesize some miRNA's are expressed differentially in WD and DD LP and that these miRNA's may be useful in identifying subtle features of dedifferentiation in a WD LP.

Design: 18 paraffin blocks with WD (n10) or DD (8) histology were retrieved from 5 WD LP and 6 DD LP. Area of WD morphology was also obtained in 4 of 6 DD LP. Total RNA was extracted from 4 x 20 µm sections for Exiqon miRNA microarrays. A pooled sample from all samples was used as common reference. Data was analyzed with SAM and Partek software to identify differentially expressed miRNAs. qRT-PCR was performed to validate selected miRNA's in the same samples.

Results: Variable numbers of human miRNA's were found to be expressed differentially between DD LP (n=6) and WD LP (n=5); tissue with DD histology (n=8) and WD histology (n=10); tissue with WD histology from DD LP (n=4) and WD LP (n=5). The down regulation of miR-539 in tissue with DD histology and miR-193a-5p in WD area from DD LP was validated by qRT-PCR.

Conclusions: The expression of specific miRNAs is different in DD LP (regardless of samples containing DD or WD histology in these tumors) compared to WD LP. Different gene expression in WD tissue from DD LP and pure WD LP has also been reported in cDNA microarray analysis.These findings suggest a role of these miRNAs in progression of LP and change in miRNA expression can occur even before or without histologic dedifferentiation. The results of this study need to be further validated in larger tumor sets. The validated results can provide guidance to identify candidate genes functioning as oncogenes or tumor suppressors in LP, and subsequently potential targets for treatment and markers for early prediction of dedifferentiation in LP.

Breast

127 Use of High Resolution Array Comparative Genomic Hybridization (aCGH) To Identify Mouse Double Minutes 4 (Mdm4) as an Early Genetic Change in Breast Cancer Development: Evaluation of Mdm4 as a New Prognostic and Predictive Marker

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Background: aCGH identified recurrent gain of chromosome 1q31-32 in >50% of BC. MDM4 gene maps to this locus. We hypothesised that it may be a candidate oncogene and tested this hypothesis on several levels: 1) Copy number alteration, 2) mRNA expression, 3) protein expression and 4) clinicopathological outcome.

Design: aCGH was performed for three independent BC series. MDM4-mRNA expression levels were assessed in 2-independent sets of gene expression arrays. Protein expression levels were assessed using immunohistochemistry in two-series of 1081 BCs with long term follow up and 140 cases of BC with matching normal terminal ductal lobular units (TDLUs) and precursor lesions.

Results: Amplification of MDM4 was detected in 15% and 8% of low and high grade BC; respectively. MDM4-mRNA expression levels significantly correlated with copy number (Pearson's correlation=0.55, p=0.0001) and this gene is overexpressed when amplified (Mann-Whitney U test p =0.0018). Mdm4 was overexpressed in 17% of BC and was associated with low grade, ER+ and normal expressions of p53, ATM and BRCA1. In cases showing coexistent precursors with invasive component, MDM4 expression was identical in both lesions. On multivariate analysis that included NPI, MDM4-overexpression was an independent prognostic marker for patients survival outcomes [HR, 0.4; p<0.0001]. In high risk ER+ patients absence of MDM4-overexpression predicted better response to hormone therapy [HR, 2.7; p<0.0001].

Conclusions: Mdm4 is an independent prognostic and predictor of BC and its overexpression could represent a novel molecular mechanism by which a subset of BC escapes p53-dependent growth control, providing new avenues for therapeutic intervention.

128 The Interaction between Mitotic Index and Bcl2 Expression Provides an Improved Separation Method for Determining Clinical Outcome Compared to Nottingham Histological Grading System (NGS)

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Background: We hypothesised that the interaction between mitotic index (M) and Bcl2 accurately discriminates between low and high-grade breast cancer (BC) and provides a more objective measure of clinical outcome than histological grade especially for patients with small size and oestrogen receptor (ER) negative cancers**.**

Design: Two independent series of 1650 and 245 invasive BC with long term follow up were subjected to immunohistochemical analysis with antibodies against apoptosis and cell cycle-related proteins. Mitotic index (M) was assessed according to Nottingham Grading System (NGS): M1: <10 mitoses; M2: 10 to 18 mitoses; M3: > 18 mitoses. Subsequently, BC were classified according to the combined M/Bcl2 profile and compared to NGS.

Results: In multivariate Cox regression models including validated prognostic factors, the subgroups defined by M/Bcl2 profile performed better than lymph node status and tumour size. Incorporation of the M/Bcl2 profile into the Nottingham Prognostic Index (NPI) accurately reclassified twice as many patients into the excellent prognosis group, improving decision-making for which patients should be spared systemic adjuvant therapy. Patients with M2-3/Bcl2- and M3/Bcl2+ (high risk) had a 2-3 fold increase in the risk of recurrence when treated with either adjuvant hormone therapy or anthracycline-based chemotherapy than those with M1/Bcl2± and M2/Bcl2+ (low risk) (HR= 3.4 (2.8-5.6); p<0.0001 and HR=2.3 (1.2-4.3); p=0.0009).

Conclusions: In conclusion a grading system defined by mitotic counting and Bcl2 expression accurately reclassified patients with NGS-G2, small size cancers or ER negative into two groups: low risk (NGS-G1 like) versus high risk (NGS-G3 like) of both BC mortality and recurrence, improving prognosis and therapeutic planning.

129 A New Morphological and Genetic Map for the Evolutionary Pathway of Low Nuclear Grade Breast Neoplasia (LNGBN) Family

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Background: There is evidence to suggest that tubular (TC) and lobular carcinoma (ILC) and their putative precursor lesions: columnar cell lesions; CCLs, low grade

ductal carcinoma in-situ (DCIS) and lobular neoplasia (LN) may consist in a family of interrelated lesions.
Design: To identify molecular genetic profile and potential evolutionary pathogenetic pathways of this family, 15 of TC an ILC and their matched coexisting CLLs, DCIS and LN were laser-microdissected. The isolated non-amplified-DNA subjected to array-Comparative Genomic Hybridization (32K BAC-array platform). Results were validated using, gene expression array, fluorescent (FISH) and chromogenic (CISH) in situ hybridization and immunohistochemistry. Comparative analysis was performed with aCGH dataset derived from 171 unselected series of BC.
Results: We observed that lesions from the same patient displayed remarkably similar patterns of genetic aberrations (Spearman's correlations 0.55-0.89; $p<0.00001$). All CLLs, low grade DCIS, LN and their matching invasive carcinoma harboured gain/amplification of 1q31-32 and loss of 16q. In addition in situ and matching invasive components displayed additional genetic aberrations at one of 16p13.3, 11q13.1-q14.1, 17q25.3, 19p13.3. Amplification of cyclin D1 was confirmed as a target gene by mRNA gene expression and CISH. MDM4 gene on 1q32 was confirmed as an early genetic change using gene expression array and IHC.
Conclusions: Our results confirm that 1) Tubular land lobular carcinoma consists in a family of LNGBN, 2) CLLs are early non obligate precursor components of the LNGBN family, 3) Cyclin D1 and MDM4 are among oncogenes that lead to activation of luminal pathway and progression of LNGBN family.

130 Estimation of Risk of Recurrence of Early Stage Estrogen Receptor Positive (ER+) Breast Carcinoma (BC) by Pathologists Compared to Oncotype Dx Recurrence Score – A Multi-Institutional Study

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Background: The Oncotype Dx assay is increasingly being used to predict recurrence in early stage BC; however, it has not been adequately compared to prediction based on traditional clinicopathologic features.
Design: We selected 153 patients with early stage ER+ BC and available Oncotype Dx recurrence score (RS). Clinicopathologic data, including age, menopausal status, tumor size, grade, mitotic activity, lymphatic invasion (LVI), nodal status, hormone receptor and HER2 status on all patients were provided to 21 breast pathologists. Participants were asked to estimate the risk of recurrence based on clinicopathologic data and provide the three most important tumor features their estimates were based on. Risk estimates of participants were compared with RS results.
Results: Based on Oncotype Dx results, 94 (61.4%), 45 (29.4%) and 14 (9.2%) BC were low (RS <18), intermediate (RS 18-30) and high (RS ≥31) risk, respectively. RS values showed significant correlation with tumor grade, mitotic activity, LVI, hormone receptor and HER2 status, while no correlation with age, menopausal status, tumor size and histologic type was found. Participants' risk estimates agreed with the Oncotype Dx assay in 52.5 ± 2.7 % (mean ± SEM, range 29.4 - 71.9) of cases, while the risk was over- and underestimated compared to RS results in 32.6 ± 3.6 (range 8.5 - 64.1) and 15.0 ± 1.4 (range 6.5 - 28.1) %, respectively. The rates of overestimation were significantly higher than those of underestimation ($p = 0.0002$). Assessment of the concurrence of participants with RS results showed a mean kappa value of 0.27 (range 0 - 0.53). The intraclass correlation coefficient for agreement for all participants was 0.501. Participants ranked tumor stage/nodal status, histologic grade and tumor size as the most important clinicopathologic features for estimating recurrence risk.
Conclusions: Compared to Oncotype Dx, pathologists tend to overestimate the risk of tumor recurrence based on traditional clinicopathologic features alone. While the RS may provide additional information regarding intrinsic biological features of ER+ BC, development of a nomogram to reliably predict RS from available clinicopathologic data would be useful and economical in patient management.

131 Significance of Partial Reverse Cell Polarity in Breast Cancer (BC) – Part of a Spectrum of Micropapillary (MP) Carcinoma?

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Background: Invasive MP carcinomas (IMPC) of the breast are aggressive tumors frequently associated with lymphatic invasion (LVI) and metastasis even when MP features are very focal. We have noticed that some agressive BC showing lymphatic spread have occasional tumor cell clusters reminiscent of IMPC without the characteristic prominent retraction artifact seen in IMPC.
Design: We prospectively selected 1323 invasive ductal BC for the study. All H&E slides were reviewed and the presence and extent of MP differentiation and retraction artifact were determined. One representetative block per case was used for immunostaining for EMA. Partial reverse cell polarity (PRCP) was defined as prominent linear EMA reactivity on at least part of the periphery of tumor cell clusters usually associated with decreased cytoplasmic staining. The clinicopathologic features of BC with PRCP were compared to IMPC and ductal BC without this feature (IDC).
Results: Of the 1323 cases 96 (7.3%) and 92 (7.0%) showed MP features and the presence of PRCP, respectively. The results are summarized in the Table. Hormone receptor and HER2 status showed no correlation with the presence of PRCP or MP features.

Summary of results

		IDC (n=1135)	PRCP (n=92)	IMPC (n=96)	p
Age (ys)		56 (56.9±0.4)	55.5 (55.6±1.3)	59.5 (58.8±1.4)	0.193
Size (cm)		1.5 (1.9±0.1)	1.7 (2.1±0.1)	2.2 (2.7±0.2)	<0.0001
Grade (%)	Low	213 (18.8)	2 (2.2)	10 (10.4)	0.0005
	Intermediate	510 (44.9)	51 (55.4)	50 (52.1)	
	High	412 (36.3)	39 (42.4)	36 (37.5)	
Percent retraction		20 (26.2±0.8)	10 (17.7±2.6	60 (57.9±2.8	<0.0001
LVI (%)	Present	353 (31.1)	89 (96.7)	81 (84.4)	<0.0001
	Absent	782 (68.9)	3 (3.3)	15 (15.6)	
LN metastasis (%)	Present	416 (36.7)	79 (85.9)	75 (78.1)	<0.0001
	Absent	719 (63.3)	13 (14.1)	21 (21.9)	
EMA staining		255 (226.2±5.3)	200 (194.3±7.0)	100 (106.2±9.4)	<0.0001

Conclusions: The presence of PRCP in BC is strongly associated with lymphatic tumor spread even if present only focally. BC with PRCP appear to have the same implication as MP differentiation and may represent part of a spectrum of IMPC. Complete or partial reversal of cell polarity may play a significant role in lymphatic tumor spread.

132 Adequate Histologic Sampling of Breast Core Needle Biopsies (CNB) in the Era of Molecular Testing – Is More Just More?

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Background: CNB is widely used to evaluate clinically and radiologically detected breast lesions. It is current practice to examine at least 3 levels from each block of CNB samples. This recommendation was mainly based on a few studies evaluating the necessary amount of sampling to detect calcifications and adequately evaluate small foci of atypia. In the current era of molecular testing the limited amount of tissue obtained by CNB is highly valuable, especially in cases of carcinomas considered for neoadjuvant therapy. We aimed to determine the amount of sampling necessary to establish an accurate diagnosis for clinical management in a series of unselected CNB of the breast.
Design: We prospectively identified 349 consecutive CNB for the study. The indication for CNB was mass (n=237), calcifications (n=80), architectural distortion (n=20) or enhancing lesions on MRI (n=12). CNB were done under ultrasound, stereotactic and MRI guidance in 231, 106 and 12 cases, respectively. Three levels of all blocks were examined routinely. In cases with indications other than mass lesions three additional levels were obtained in each case. Histologic, clinical and radiological findings were correlated.
Results: The median number of total cores and cores with diagnostic lesion was 6 (range 1-36) and 4 (range 0-24), respectively. Diagnostic lesion or findings consistent with the clinical/radiologic impression was present on level 1 in 327 (93.7%) cases, including 235 (99.2%), 62 (77.5%), 20 (100%) and 11 (91.7%) CNB done for mass, calcification, architectural distortion and MRI findings, respectively. No mass lesion was identified in 2 CNB done for that indication in 2 (0.8%) cases despite deeper levels. In the 22.5% of cases where calcifications were missed on level 1, they were identified on level 2 in 8 (10%), level 3 in 4 (5%) and additional levels in 4 (5%) cases; no calcifications were identified in 2 (2.5%) cases even on additional levels. One (8.3%) case with MRI findings showed a focus of ADH on level 2 only.
Conclusions: Our findings suggest that examination of a single level obtained from CNB of the breast yields accurate diagnosis in the vast majority of cases, especially when CNB is performed for mass lesions under ultrasound guidance. In cases with discordant findings or when small foci of atypia are seen, additional levels should be obtained. Routine examination of a single level of CNB would reduce cost and spare valuable tissue for molecular or other studies.

133 Invasion and Cancer Stem Cell Biomarkers Can Predict Breast Cancer Metastasis

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Background: Breast cancer mortality is largely attributable to the development of systemic, hematogenous metastatic disease. Currently established prognostic criteria such as the histopathological grade of the tumor or tumor size do not successfully predict systemic metastatic potential. Enrichment of cancer stem cells in the primary tumor is a known poor prognostic marker . Epithelial to Mesenchymal Transition (EMT) in breast cancer cells has been reported to give rise to cells having cancer stem cell like properties. Both of these cells types are highly invasive. We have identified invasion markers that can predict EMT, drug and radio resistance in breast cancer cells. Here we use biomarkers for cancer stem cells ($CD44^+/CD24^-$) and markers of invasion Mena/Mena 11a to identify cancer initiating cells in Fine Needle Aspiration (FNA). We correlate these ratios with a microanatomical structure called Tumor Microenvironment of Metastasis (TMEM) comprising of a direct apposition of an endothelial cell, a cancer cell and a stromal macrophage in the breast cancer tissue. TMEM has been reported to strongly correlate with metastasis.
Design: FNA was performed on lumpectomy or mastectomy tissue using fine needle, quality was determined by smear staining. The cells were enzymatically digested, fixed, permeabilized and subjected to flowcytometry analysis using antibodies against stem cell and invasion markers. The rest of the tumor was processed for histopathology and TMEM analysis. Statistical analysis was done to identify correlation.
Results: Our initial results show that there is a strong correlation between $CD44^+/CD24^-$ cells in the FNA with TMEM scores. We also report a very high correlation between TMEM scores and the ratios of different splice variants of Mena at the message level.
Conclusions: The percentage of $CD44^+/CD24^-$ cells can be used to estimate the number of cancer stem cells in a FNA sample. The ratio of Mena/Mena 11a can be used as

a measurement of tumor cell response to the microenvironment and its metastatic phenotype. Measuring the stem cell biomarkers and Mena isoforms in cells collected by FNA biopsy using FACS and combining that with TMEM density we will be able to develop a simple, quick and inexpensive method of determining metastatic potential of an individual tumor.

134 The Concordance of Estrogen and Progesterone Receptor Status (ER-PR), and Her-2/Neu Expression in DCIS Coexistent with Micro-Invasive and Invasive Carcinoma

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Background: Determining ER, PR and Her-2/neu expression status in microinvasive carcinoma is often hampered by the lack of adequate microinvasive component on the additional tissue sections used for immunohistochemical (IHC) evaluation. Our objective was to correlate the IHC status of ER-PR and Her-2/neu in a cohort of patients with coexistent ductal carcinoma in situ (DCIS) and invasive carcinoma (IC) including a subset of patients with microinvasive carcinoma (MIC).

Design: From our pathology archives, we identified 117 consecutive patients with the diagnosis of DCIS and coexistent IC/MIC between 2004-2006. The immunostains for ER/PR and Her-2/neu were performed using established protocols and specific antibodies. For the purpose of this study, the H&E slides and the IHC stains for ER, PR and Her-2/neu receptors were reviewed with the IHC stains evaluated according to the CAP/ASCO guidelines.

Results: Our cohort included 100 patients with DCIS+IC and 17 with DCIS and MIC Ninety-nine (99%) of the DCIS+IC cases exhibited concordance between the DCIS and IC components. This was true for the 51 cases with ER/PR+, Her2 -, the 16 cases with ER/PR+ and Her2 +, the 12 cases with ER/PR- and Her2+ and the 21 cases with ER/PR- and Her2 -. While the ER-PR were concordant in the last case, Her-2/neu was negative in the DCIS and positive in the invasive tumor. Only 7 of the 17 patients with DCIS and MIC tumors had adequate MIC component for IHC evaluation with 100% concordance.

Conclusions: Accurate determination of ER-PR and Her2 neu status is an essential component of the pathological evaluation of mammary neoplasia with major management implications. Our data show a 99% concordance between the DCIS and IC and 100% concordance between DCIS and MIC. Despite the smaller number available in the MIC category, our results suggest that the minimally invasive disease is likely to mirror the ER-PR and Her2 neu status of its coexistent DCIS.

135 Molecular Classification of Male Breast Cancer Using Immunohistochemistry

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Background: Male breast cancer is uncommon, accounting for 0.8% of all breast cancers, but is important given it's association with BRCA1 mutations. A recent gene expression study using RT-PCR showed similar molecular subtypes in male breast cancer as seen in female breast cancer. The purpose of this study is to characterize the molecular subtype of male breast cancer using a panel of immunohistochemical stains.

Design: The records of 25 male breast cancer patients, mean age = 67.7 years (range, 47-84 years) were gathered from two institutions, Palo Alto Veterans Affairs Health Care System and Stanford Hospital. We performed immunohistochemical (IHC) staining for the following antibodies: ER, PR, HER2, EGFR, CK5/6, p63, and calponin. Slides were scored by two observers.

Results: All cases were invasive ductal carcinoma, with two cases also containing an in situ component, as confirmed by intact myoepithelial cell staining by p63 and calponin stains. Staining results and molecular classification are shown in Table 1. Of the 25 cases, 20 (80%) were classified as luminal subtype based on ER reactivity, and lack of HER2, basal cytokeratin, p63 or EGFR staining. Four (16%) were characterized as HER2 subtype based on positive staining results for HER2. One (4%) was classified as basal subtype given a triple negative, basal cytokeratin-positive, p63-positive and EGFR-positive phenotype.

Table 1. Immunohistochemistry Results

ER	PR	HER2	EGFR	CK5/6	p63	Molecular type
20/20	18/20	0/9	2/19	0/19	0/19	Luminal
4/4	3/4	4/4	0/3	0/3	0/3	HER2
0/1	0/1	0/1	1/1	1/1	1/1	Basal

Conclusions: To the best of our knowledge, this is the first study attempting molecular subtyping of male breast cancer using an immunohistochemical panel approach. We have shown that similar molecular subtypes are seen in male breast cancer as in female breast cancer, in approximately similar frequencies. As molecular subtyping and individualized medicine become more prevalent, this simple and cost effective IHC analysis may provide more efficient classification of male breast cancer.

136 Analysis of Osteopontin Expression in HER2-Positive and Basal/Triple Negative Breast Carcinomas

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Background: Osteopontin (OPN) is a multifunctional protein found in a variety of normal and neoplastic tissues. A few recent studies in breast carcinoma (BC) have suggested an association with adverse pathological and poorer clinical outcome. The aim of our study was to evaluate OPN expression in a series of BC with an aggressive phenotype, such as HER2-positive and basal/triple-negative (TN) to determine its prognostic value.

Design: OPN expression was studied immunohistochemically (IHC) on paraffin-embedded tissue micro-arrays of 234 BC: 142 (60.7%) HER2-positive (3+ in >30% cells by IHC or amplification by FISH/CISH), and 92 (39.3%) basal/TN (ER/PR/HER2-negative +/- CK5/6 +/- EGFR). OPN cytoplasm staining was semiquantitatively scored based on intensity (0-3+) and distribution (0-100%) (score 0-300). The results were correlated with clinico-pathological variables and patients' outcome. Data was statistically analyzed using Chi-square or Fisher's exact tests, and survival was calculated by the Kaplan-Meier method (log-rank test).

Results: Median patients' age was 56 years (range 28-87 years) with a median follow-up of 65 months. In this series, tumors were more frequently >20 mm (54%) in size, of grade 3 (72%), and lymph-node status negative (56%). OPN overexpression (score >50) was observed in 33.5% BC. Among them, were found those presenting in older patients (84%; p=0.019), of high grade (83%; p=0.044), with positive lymph-node status (65%; p=0.042) and basal/TN immunophenotype (76%; p<0.000). However, no correlation was observed for tumor size, the presence of necrosis or vascular invasion (p=ns). Survival analyses stratified by immunophenotypes demonstrated that HER2/OPN-positive patients compared with those with HER2/OPN-negative, had a trend towards poorer disease-free (25% vs 61%; p=0.11) and overall survival (40% vs 73%; p=0.058), but no differences were seen for the basal/TN/OPN-positive group (p=ns).

Conclusions: In the current series of BC, OPN overexpression was related with unfavorable prognostic factors, and a negative impact on survival in patients with HER2-positive tumors. Thus, our results support that OPN is a potential marker of poor prognosis and it may represent an attractive target of therapy.

137 A Limited Number of Sentinel Nodes Accurately Stages the Axilla

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Background: Axillary lymph node status is the single most important prognostic factor in patients with breast cancer. Over the past decade, sentinel lymph node biopsy (SLNB) has become the standard of care for axillary staging. However, limited studies address the question of how many sentinel nodes need to be biopsied to ensure adequate screening, especially in the era of pre-screening with axillary ultrasound and ultrasound-guided axillary lymph node sampling.

Design: We reviewed all cases of positive sentinel lymph node biopsy during a four year period to determine how many nodes needed to be sampled for accurate staging. During this time, all patients at the University of Chicago Medical Center were pre-screened with axillary ultrasound and, when warranted, underwent ultrasound-guided biopsy. A positive biopsy alleviated the need for SLNB and, hence, these patients were not considered in our study.

Results: We identified 348 patients who underwent SLNB (2004-2008) at the University of Chicago Medical Center. SLNB identified carcinoma in the axillary nodes of 67 of these patients. In all, 191 lymph nodes were recovered from this group (range 1-6; mean 2.8). The excison of the first sentinel node was sufficient for a positive diagnosis in 76% of these cases, excision of a second node increased the capture rate to 91%, while a third node increased it to 96%, and removal of a 4th node captured all patients with a positive sentinel node. Interestingly, two patients with nodal disease had negative sentinel nodes, but positive non-sentinel nodes palpated and excised by the surgeon during SLNB. During this same period of time, 281 patients underwent SLNB with no evidence of metastatic disease in the axilla. In all, 766 lymph nodes were recovered from this group (range 1-11; mean 2.75).

Conclusions: Excision of four sentinel nodes accurately identified all cases of positive sentinel node in our study. Excision of greater than 4 nodes does not lead to additional prognostic information and instead consumes valuable anesthesia, surgeon, and pathologist time while providing limited benefit to the patient. Larger studies are necessary to further support this conclusion.

138 Can Pathologic Features and Immunophenotype of ER+, Node Negative Breast Cancers Identify High and Low Risk OncotypeDX Subgroups?

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Background: The NCCN guidelines currently give consideration to OncotypeDX testing of ER positive, lymph node negative invasive breast cancers to help predict benefit from chemotherapy. However, the test is expensive ($3500) and only those cases resulting in high or low recurrence scores (RS) can affect treatment choices. This study was designed to assess whether pathologic and immunophenotypic characteristics can accurately identify those tumors with either high or low RS.

Design: We retrospectively reviewed 104 cases of ER-positive, node negative breast cancers that had been sent for OncotypeDX testing. All cases were assessed for Nottingham grade as well as semiquantitative immunohistochemical analysis of ER and PR (Allred scores), Ki67-Ag, HER2, cyclin D1, bcl-2, p53 overexpression, and lymphatic invasion (tumor in podoplanin-positive spaces). Analysis was then performed to determine if there were immunophenotypically defined patient subgroups corresponding to the high and low RS subgroups.

Results: Overall, 44 (42%) of cases had low RS, 49 (47%) had intermediate RS and 11 (11%) had high RS. Of 85 ductal carcinomas 9 were high RS, 38 intermediate and 28 low RS. Low grade was significantly associated with low RS ($p = 0.0024$) and high grade was associated with high RS ($p = 0.032$). PR > 5 was strongly associated with low RS ($p < 0.0001$) and PR< 5 was associated with high RS ($p = 0.0027$). Using a combination of histologic type, grade and PR levels, we identified subgroups of patients with highly predictable RS. 86% (18 of 21) grade 1, PR > 5 ductal carcinomas were low RS and 0 were high RS. In contrast, 83% (5 of 6) grade 3, PR < 5 ductal carcinomas had high RS and 0 were low RS. There was also a strong association between high RS tumors and the presence of lymphatic invasion and p53 overexpression.

Conclusions: Subgroups within ER positive, lymph node negative breast cancers can be defined by tumor grade and immunophenotype that correspond to high and low RS defined by OncotypeDX testing. Determination of these histologic and immunophenotype-defined subgroups could have accurately subclassified 23% of the total cases and avoided $84,000 of OncotypeDX testing.

139 Invasive Lobular Carcinoma of the Breast. A Clinicopathologic Study of 1,200 Cases

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Background: Invasive Lobular Carcinoma (ILC) account for approximately 5% to 15% of invasive breast carcinomas and are the second most common type. As such, most clinical research conclusions are driven by the outcome of patients with Invasive Ductal Carcinomas(IDC), which dominates the breast cancer population. The goal of this study is to investigate the clinical and pathological features of ILC.

Design: From 1998 to 2008, 5200 patients with invasive breast cancer were identified at our Institution. The clinical and biological features of the patients with IDC were compared with those patients with ILC. The median follow-up period was 72 months. Clinical variables were compared using Chi square and Fisher´s exact tests.

Results: One thousand and two hundred (23%) tumors were classified as ILC and 4000 (76.9%) as IDC. In comparison with IDC, ILC was significantly more likely to occur in older patients, to be larger in size (2cm vs 4cm, respectively), to be estrogen and progesterone receptor positive and to be HER-2 negative (P:<.01). Most ILC cases were grade 2 tumors(80%), while a small proportion of tumors were either grade 1 or 3 tumors(10% each). The incidence of contralateral breast cancer was higher for ILC patients than for IDC patients (P: < 0.1). The 5-year overall survival was 70.2% for ILC and 68.3% for IDC (P:0.66).

Conclusions: ILC is a distinct entity of breast cancer, on the other hand, patients with this type of tumor do not have better clinical outcome than patients with IDC.

140 Immunohistochemical Identification of a Metastasis Modifier Gene (Rrp1b) in Breast Cancer May Help Identify Tumors with High Metastatic Potential

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Background: Breast cancer is the second commonest malignancy in women and most breast cancer related mortality is a consequence of metastasis. A metastasis modifier gene, called signal induced proliferation-associated gene 1 (Sipa-1) has been recently identified in breast tumor cells and its expression has been demonstrated to correlate with increased metastatic capacity. Sipa-1 and a group of related genes constitute the diasporin pathway. Among them, Ribosomal RNA Processing 1 Homolog B **(**Rrp1b) gene, which directly interacts with the Sipa-1 gene, plays an important role in metastatic predisposition. Over-expression of Rrp1b induces a gene expression signature that predicts survival in *in-vitro* mammary tumor cells**;** however, its expression in human breast cancer specimens and its value as a predictive marker of metastatic disease have not been previously investigated *in vivo*.

Design: Protein expression of Rrp1b gene was evaluated by immunohistochemistry in 54 primary and 17 metastatic breast cancers which included metastases to 11 lymph nodes, 6 lungs, 2 brains and 6 livers. Tissues were scored as positive for Rrpb1 when there was cytoplasmic and/or membranous staining. The staining intensity (weak, moderate or strong) and distribution (diffuse or focal) were noted. The results were correlated with the clinico-pathological data of the patients including prognostic factors.

Results: Overall, positive staining for Rrp1b in the primary breast tumors was seen in 64% of the cases, 67% of the metastatic lymph nodes, 67% of lung metastases and all metastases to the liver and brain. Positive staining was observed in 94% of the infiltrating ductal carcinoma and in none of the infiltrating lobular carcinoma. Strong staining was seen in the metastatic and infiltrating ductal carcinoma, but weak in the ductal carcinoma in-situ cases. Positive Rrp1b staining was seen in 96% of the tumors with positive lymph nodes. Eighty-five per cent of the ER negative cases were positive for Rrp1b (p=0.042). Tumors negative for ER, PR and c-erb2 expressed Rrp1b (p< 0.0001). All tumors >4cm were positive for Rrp1b.

Conclusions: Our results demonstrate for the first time that Rrp1b is indeed expressed in human breast cancers *in vivo*. Rrp1b expression correlates with clinical stage, hormonal markers and HER-2 amplification. Rrp1b can hence be used as a potential marker of metastatic susceptibility and a possible marker for the development of molecular targets and therapy.

141 Coronal Serial Sectioning of Lumpectomy Specimens: Proposed Technique of Sectioning and Submission of Tissue for Microscopic Examination of Breast Carcinoma

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Background: Lumpectomy specimens are currently sectioned in a plane perpendicular to the long axis of the sample and skin (random serial sectioning technique -RSS). It is often necessary to correlate areas of tumor with radiological findings, which is often difficult with the RSS technique. We hypothesized that coronal sectioning has better reproducibility and might be a better alternative.

Design: Fifty lumpectomy specimens for breast carcinoma were sectioned in the coronal plane (parallel to the skin/chest wall) at 3-5 mm intervals (coronal serial sectioning technique - CSS). All giant sections of the breast tissue between the superficial and deep margins were divided in a "grid" pattern into routine sections. The superficial and deep margins were cut in the plane perpendicular to the coronal plane. For large specimens, the findings of this protocol were compared with two modified protocols involving fewer sections: *Modified protocol 1*) for cases with diffuse fibrosis or cystic changes (10 cases), findings on microscopic examination were recorded from entire alternate "giant" cross sections; *Modified protocol 2*) for cases consisting predominantly of adipose tissue (3 cases), findings of microscopic examination were recorded from fibrotic areas. Additional sections were required for areas suspicious for margins involved by carcinoma.

Results: CSS demonstrated DCIS and invasive carcinoma in its largest area and along the greatest diameter as opposed to the RSS that divided the greatest diameter of the breast lesion in multiple sections. Findings on status of DCIS, resection margins and multi-centricity, and dimensions of DCIS and invasive carcinoma were similar in three protocols of CSS for large specimens. But CSS had the following benefits over RSS: a) easily reproducible panoramic view of different areas and types of neoplasms in the sample, b) accurate measurement of tumor size, c) feasibility for reconstruction of the breast specimen to re-examine areas of interest after the initial microscopic examination, and d) no requirement of special equipment, extra-time of fixation or time-consuming training to achieve better results in comparison with conventional RSS.

Conclusions: We propose that CSS is more scientific and a better approach for processing lumpectomy samples of the breast.

142 Amplification of the Oncogen HER2 by Immunohistoquemistry and FISH in Patients with Breast Cancer in Colombia. Analysis of 2879 Consecutive Cases Comparing Peripheral Laboratories and a Reference Institution

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Background: Studies for the detection of the HER2 status are mandatory in every patient with a diagnosis of invasive breast cancer due to its implications as a prognostic and predictive factor for the response to Trastuzumab therapy.

Design: 2879 consecutive cases referred for the determination of the HER2 status by FISH and/or IHQ were retrieved from the data base. The analysis included correlation between the IHQ HER2 score submitted by the peripheral laboratory (PL) and the FISH studies performed in the central laboratory (CL), comparison between de Herceptest Score and the copy numbers by FISH, comparison of the HR results and the HER2 status by IHQ and FISH.

Results: From the total number of cases 2127 HER2 IHQ studies were performed in the (CL), using the Dako Herceptest ; the results are : 67,6 % were negative score 0-1+; 14,6% were indeterminate (2+) ASCO/CAP 2008; 15,6% were 3+ and 2,1 % were not adequate. 1079 cases were studied by FISH (37,5%), 905 shown adequate hybridization signals.83,8%. 631 (69,7%) came from the (PL), and 276 (30,3%) from the (CL). The correlation between the HER2 score performed in the (PL), was 10/41 cases 0-1+ had amplification by FISH corresponding to 24,4,%. 258/529 (2+) 48,7% and 52/61(3+) 85,2% were FISH(+) . In comparison when both studies were performed in the (CL). 1/9 cases HER2 (0+) 11,1% 3/17 HER2(1+) 17,6%,139/241HER2 (2+)57,6% and 9/9 HER2(3+) 100% were FISH positive, from the,only 1/4 cases negative (score 0 and 1) corresponded to an in-house case. (3,8% of false negative).The range of FISH amplification were: 2.65-4.8 for the (0+); 2,2-7,5 for the (1+); 2,2-15.2 for the (2+) and 3,5-14,5 for the (3+). In 280 of the cases studied by FISH the results of the HR. were available, 45,8 of the FISH(+) were RH(+). In 824 of the HER2 cases studied by IHQ the HR were positive in 70,9% of (0+); 84,2 (1+); 71% (2+) and 37,9% o (3+).

Conclusions: Similar to what has being reported in developed countries the false negative and false positive results of the HER2 by IHQ in peripheral laboratories is unacceptable high in Colombia with a false negative rate of 24,4% and a false positive of 14,8%. A very high number of tissue blocks submitted for FISH studies were not adequate, indicating that preanalytical factors have to be improved in Colombia in order to get optimal tissue for molecular studies speciality in the era of the molecular targeting therapies.

143 Sentinel Node (SN) Mapping in High Grade Ductal Carcinoma In-Situ (HG-DCIS)

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Background: The decision to performed SN mapping in HG-DCIS patients undergoing breast conservation remains controversial. Currently many breast cancer centers have adopted this practice based on archival data showing up to 20% incidence of axillary metastasis. This retrospective study correlates key pre-operatory mammographic findings with the incidence of invasive carcinoma (inv-ca) and metastatic disease on final surgical specimens and provides criteria for planning SN mappings in patients with this disease.

Design: Imaging data from 130 patients presenting with mammographically suspicious microcalcifications (Ca++) and Needle Core Biopsy (NCB) proven HG-DCIS, was reviewed for the following: morphology of Ca++ (linear, segmental, clustered, pleomorphic, amorphous, casting); extent of Ca++ (mm); associated parenchymal changes (masses, densities, asymmetries) and correlated with their final diagnosis and axillary status. All patients undergoing simple mastectomies had SN. Patients undergoing lumpectomies had SN only if Ca++ were associated with parenchymal abnormalities and/or at surgeon discretion.

Results: The incidence of inv-ca in patients with mammographic findings of suspicious Ca++ alone (n=91) and extending up to 5 cm. was none; microinvasive (m-inv) disease was present in 5 SN (-) patients. Parenchymal changes with Ca++ were noted in 39 patients: ill defined masses (n=23), increased densities (n = 9) and parenchymal asymmetries (n=7) all associated with Ca++ extending up to 2 cm. In this group, 9 patients had inv-ca and 6 had m-inv disease. Of the ones with inv-ca, 8 had associated ill defined masses, and one had increased parenchymal density pattern; 5 were SN (-), 3 were single (+) SN and one had 4 (+) nodes. Of the ones with m-inv disease, 4 were associated with ill defined masses and 2 with increased parenchymal densities; 5 patients were SN (-) and 1 had a single (+) node. No particular Ca++ morphology or pattern

correlated best with the incidence of microinvasive disease or inv. ca. No correlation between extent of Ca++ (up to 5 cm) and incidence of inv-ca was identified.
Conclusions: The management of the axilla in HG-DCIS patients undergoing breast conservation should be planned emphasizing on the patients mammographic findings: For patients with Ca++ alone, SN biopsy is not indicated. On contraire, SN mapping is indicated in patients with suspicious Ca++ associated with parenchymal changes, particularly if the changes are ill defined masses, even if the NCB shows no evidence of inv-ca.

144 From the Bin to the Gene: Leftovers of Diagnostic Fine Needle Aspiration Cytology Can Be Successfully Used To Perform Gene Expression Profiling of Breast Carcinomas

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Background: Molecular testing of breast cancer is emerging as an ancillary technique to conventional histopathological tools. Several predictive signatures based on differential expression of genes between distinct tumour groups have been described so far and one of these has recently gained FDA approval.The success of molecular tests is strictly related to an adequate sampling of the lesion and to the good preservation of material to be subjected to the analysis. Therefore, it is mandatory to use fresh samples that are representative of and enriched for the tumour cell population in order to obtain a sufficient yield of good quality RNA. Our aim was to investigate whether leftovers from diagnostic Fine Needle Aspiration (FNA) cytology can be successfully used to extract RNA of enough quantity and quality to be subjected to different types of molecular analysis.
Design: We collected 28 samples of FNA leftovers obtained from mammograpically detected opacities. FNAs were performed under ultrasound guidance using a multi-pass approach with a 22-gauge needle attached to a syringe in a pistol-grip holder. The aspirated material was immediately smeared and stained, then the syringe was washed in Trizol solution and the collected material was transferred in vials at 4°C. Following RNA extraction and quantification, RT-PCR for PGK and CK-19 genes were performed. Twelve samples with different RNA concentration were selected for microarray gene expression analysis, which was performed by using the HumanHT-12_V3 Expression BeadChip by Illumina.
Results: RNA was successfully extracted from all FNA samples with a mean RNA yield of 11.7 µg/FNA (range: 0.78-88.4 µg; median: 4.85 µg; mode 7.5 µg). RNA integrity and absence of DNA polymerase inhibitors were assessed by the percentage of samples positive for PGK housekeeping gene. Twenty-six out of 28 (92.8%) samples were RT-PCR positive for PGK. These 26 samples were tested for CK-19 gene expression and all of them were positive. Microarray gene expression analysis of selected cases demonstrated a good performance in all of the 12 samples.
Conclusions: FNA cytology leftovers can be successfully used to extract RNA of enough quantity and quality to reliably perform molecular analyses.

145 Are Histologically Benign Papillarly Lesions of the Breast Associated with Increased Risk for Developing In Situ or Invasive Carcinoma? A Follow-Up Study of 302 Patients

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Background: There is abundant evidence that atypical papillary lesions of the breast are associated with a significant risk of carcinoma and need to be excised. The clinical significance of diagnosing a 'benign" papilloma is controversial. The objectives of this study was to define the risk associated with papillomas and atypical papillomas in breast biopsy specimens.
Design: Using our institutional database, we identified 415 consecutive breast biopsies indicating the presence of a papillary lesion between 1997 and 2000. Follow up data were obtained from our institutional record and the national SEER registry. The cases were classified into three pathological categories: benign papillomas with no atypia, atypical papillomas (papillomas with atypical architectural/cytological features or papilloma with coexistent atypical ductal hyperplasia), and papilloma with coexistent DCIS. Statistical correlation of these categories with patient follow up was determined using Chi-square test.
Results: Three hundred and two of the 415 papillary lesions (75%), had histologic follow up with subsequent breast sampling. Mean age at diagnosis was 53 years (19-93 years). The median follow-up was 106 months (0-155 months). Two hundred and fifty six cases were classified as benign papilloma with no atypia and 29 as atypical papilloma. Seventeen patients who had papilloma with coexistent DCIS served as control. Overall, on follow-up, 17 of the 302 patients (5.6%), were diagnosed with ductal carcinoma in situ (8 cases) or invasive carcinoma (9 cases). For the 17 patients with papilloma and concurrent DCIS initially, 5 (29%), showed persistent DCIS with a subset harboring invasive carcinoma on follow up sampling. DCIS and /or invasive carcinoma was identified on follow up in 2 of 29 (7%) patients with the initial diagnosis of atypical papilloma and in 10 of 256 (3.9%), patients who were initially diagnosed with benign papilloma. (p=0.005).
Conclusions: Our data suggest that the increased risk for developing DCIS/invasive cancer associated with atypical papillary lesions of the breast extends to patients with papilloma without atypia.

146 Mutations in PIK3CA Lymph Node-Positive Breast Carcinomas Are Associated with Luminal Immunophenotype

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Background: PIK3CA activating mutations have been identified in a high proportion of breast carcinomas (BC) (~25%). Mutations can activate the PI3K/Akt pathway and contribute to tumor progression. Its association with pathological markers is often contradictory. The purpose of the study was to evaluate PIK3CA mutations in a series of lymph node-positive (LNP) infiltrating BC and to correlated the results with immunohistochemical (IHC) variables and BC subtypes.
Design: A total of 391 LNPBC patients included in the GEICAM 9906 clinical trial were studied. IHC was applied on tissue microarrays for ER (cut-off 10%), PgR (cut-off 10%), Ki67 (cut-off 15%), p53 (cut-off 20%), HER2 (all 2+ and <30% 3+ confirmed by dual-CISH), cyclin D1 (cut-off 30%) and basal cytokeratins (CK) 5/6 and 17 (cut-off 10%). Tumors were classified according to the immunophenotype as luminal (ER and or PR ≥10%, HER2-negative), HER2-positive and triple-negative (ER/PgR/HER2-negative). For PIK3CA mutation, DNA was extracted from formalin-fixed, paraffin-embedded tissues using standard methods. We analyzed by allelic discrimination based on real-time chemistry TaqMan MGB probes in ABI Prism 7500 Sequence Detection System (Applied Biosystems). Significant associations were identified using Chi-square and Fisher's exact test. A p-value <0.05 was considered significant.
Results: PIK3CA mutations were observed in 24.6% tumors. In relation with IHC variables, 76.7% tumors were positive for ER,63% for PgR, 35% for HER2, 31.3 % for p53, 6.4% for EGFR, 68.9% for cyclin D1, 13.5 % for CK 5/6 and/or CK17) and 43.1% high Ki67. Tumors with PIK3CA mutations were more frequently positive for ER (p=0.008) and PgR (p=0.003), with low Ki67 (p=0.04), and negative basal CKs (p=0.005) and luminal phenotype (p=0.036).
Conclusions: Our findings in a series of LNPBC confirm that PIK3CA mutations are associated with luminal phenotype, low proliferative activity with Ki67 and negativity for basal cytokeratins. Supported by grant FIS 06/1488

147 Careful Radiology Pathology Correlation in Breast Biopsies with Lobular Neoplasia Aids in Triaging for Lumpectomy or Observation

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Background: Lumpectomy for noninvasive lobular neoplasia on core biopsy is controversial. Lobular carcinoma in situ (LCIS) and atypical lobular hyperplasia (ALH) are usually incidental findings, yet, some studies suggest that these findings alone on core bioppsy are associated with an increased incidence of more aggressive lesions on lumpetcomy. At our institution the majority of core biopsies with ALH or LCIS diagnoses are excised, giving an excellent opportunity for a retrospective review to assess the need for reflexive excision in the setting of detailed radiographic and pathologic correlation.
Design: 52 breast core biopsies with a diagnosis of ALH or LCIS only were used (cases with concurrent atypical ductal hyperplasia or worse were excluded). All authors were blinded to the excision results. Histology and imaging were reviewed simultaneously with the pathologist and radiologist discussing imaging abnormalities and histologic correlates. If the lobular neoplasia was questioned to be ductal, E-Cadherin immunohistochemistry was utilized. All cases were then classified as "excision warranted" or "observation only". Pleomorphic lobular carcinoma and florid LCIS (greater than 10 lobules with LCISon core) were placed in the excision group since some have speculated these have an aggressive biologic potential. After review of all images and core biopsy histology, the excsions results were unblinded.
Results: Of the 53 cases 43 had excisions. **37** cases had concordant imaging and histology that warranted observation only. 30/37 had subsequent excisions; 4 had atyipical ductal hyperplasia but none had carcinoma on excision. **6** cases were discordant radiographically (1 secondary to BiRads 5 interpretation, 2 due to lack of explanation for calcifications and and 3 due to no explanation for a mass); 2/6 had DCIS on excision. **4** cases had changes in histology (one to cancerization of the lobule by DCIS and 3 with flat epithelial atypia) and the first case had DCIS on excision. **6** cases had either pleomorphic LCIS or florid LCIS.
Conclusions: When detailed radiographic and histologic correlation is made and an explanation for the radiographic findings found, core biopsies with ALH or LCIS can be safely managed with observation. Core biosies with ALH and LCIS but otherwise bengin biopsies require detailed histologic findings so that accurate radiographic correlation can ensue, particularly quantity of microcalcifications and etiology for a mass.

148 The New Hormone Receptor Cutoff: For Defining Hormone Receptor Positivity What Is the Impact of the New 1% Cutoff Versus the Old 10% Cutoff on Hormone Receptor Concordance between Local IHC, Central IHC and Central RT–PCR?

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Background: Accurate assessment of hormone receptors (HR) in breast carcinoma is important. It is recommended that >1% should be used as the standard cutoff to define HR positivity (CAP 2009). To characterize the effect that this has on the concordance between laboratories and methods, we examined HR concordance between local and central IHC and central RT-PCR using clinical trial breast cancers from E2197.
Design: Tumors from 761 E2197 pts were examined. ER and PR results were obtained by: local IHC (reported by the site), central IHC (1D5 and 636 performed in duplicate on 1.0 mm core TMAs using Allred scores of >2, the traditional cutpoint (corresponding

to either 10% of cells staining 1+ or 1% of cells staining 2+) & the new cutoff of ≥2 (1% of cells 1+ positive)); and, by central quantitative RT-PCR analysis using Onco*type* DX (RNA extracted from formalin fixed paraffin embedded tissue, pre-defined cutoffs of 6.5 for ER and 5.5 for PR).

Results: Results from local IHC (761 pts) were compared with central IHC (755 pts) and RT-PCR (761 pts). Using a central IHC cutoff of AS>2 the concordance between central and local IHC was 90% for ER, 84% for PR and 90% for HR, while for central IHC and central RT-PCR it was 93% for ER, 90% for PR and 93% for HR. Using a central IHC cutoff of AS≥2 the concordance between central and local IHC was 91% for ER, 84% for PR and 90% for HR while for central RT-PCR it was 94% for ER, 89% for PR and 93% for HR.

Conclusions: The new HR cutpoint had a negligible impact on ER and PR concordance between central and local IHC and between central IHC and central RT-PCR. A standardized cutoff value of 1% may be easier to uniformly apply and concordance studies to examine the impact of this new cutoff are warranted.

% Concordance (95% CI)

Comparison	ER	PR	HR
Local IHC vs Central IHC (AS>2)	90 (88, 92)	84 (82, 87)	90 (87, 92)
Local IHC vs Central IHC (AS≥2)	91 (88,93)	84 (81,86)	90 (87,92)
Local IHC vs RT-PCR	91 (89, 93)	87 (85, 90)	91 (89, 93)
Central IHC (AS>2) vs RT-PCR	93 (91, 94)	90 (88, 92)	93 (91, 95)
Central IHC (AS≥2) vs RT-PCR	94 (92,95)	89 (87,92)	93 (91,95)

149 ER and PR Discordances: Comparison of IHC by Local and Central Laboratory and RT-PCR by Central Laboratory in ECOG Breast Cancer Study 2197

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Background: Accurate laboratory assessment of hormone receptors (HR) in breast carcinoma is therapeutically important. To characterize the nature of the discordances between laboratories and methods, we examined discordances for ER and PR between local IHC, central IHC and central RT-PCR using clinical trial breast cancers from E2197.

Design: Tumors from 761 pts enrolled in E2197 were examined; pts had 0-3 positive nodes and received doxorubicin and cyclophosphamide or docetaxel plus hormonal therapy. Blinded ER and PR results were obtained by local IHC (reported by the site), by central IHC (using 1D5 and 636 performed in duplicate on 1.0 mm core TMAs using pre-defined Allred score >2 cutoff); and by central quantitative RT-PCR analysis using Oncotype DX (RNA extracted from formalin fixed paraffin embedded tissue, using pre-defined cutoffs of 6.5 for ER and 5.5 for PR).

Results: Results from local IHC (761 pts) were compared with central IHC (755 pts) and RT-PCR (761 pts). The concordance for determination of hormone receptor status between local or central IHC and central RT-PCR was high and similar to the high concordance between local and central lab IHC (table). Five of 403 (2%) ER+ pts by central IHC and 21 of 429 (5%) ER+ pts by local IHC were ER- by RT-PCR; of the 25 total discordant cases between IHC and RT-PCR, 1 case was positive by both central and local IHC and the remaining 24 cases were positive by only local or only central but not by both. Fifty of 352 (14%) ER- pts by central IHC and 44 of 332 (13%) ER- pts by local IHC were ER+ by RT-PCR; of the 72 total discordant cases between IHC and RT-PCR, 22 cases were negative by both central and local IHC and 50 were negative by only local or only central but not by both.

Conclusions: These results indicate that many of the discordances between IHC and quantitative RT-PCR do not reflect pre-analytic or analytic differences between protein and RNA measurements but may be attributed to inter-laboratory variability in IHC assessment.

% Concordance (95% CI)

Comparison	ER	PR	HR
Local IHC vs Central IHC	90 (88, 92)	84 (82, 87)	90 (88, 92)
Local IHC vs RT-PCR	91 (89, 93)	88 (85, 90)	91 (89, 93)
Central IHC vs RT-PCR	93 (91, 94)	90 (88, 92)	93 (91, 95)

150 Quantitative Gene Expression by RT-PCR in the Special Histologic Subtypes of Estrogen Receptor Positive Invasive Breast Cancer

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Background: ER+ breast ca is characterized by histologic subtypes. The special histologic subtypes are prognostically significant (Rosen, 2009). We report the histologic subtypes of ER+ breast ca and associated patterns of gene expression as measured by the 21 gene assay.

Design: 100K tumors from 7/05 thru 5/09 were included. Tumors were classified by histologic subtype using WHO criteria (IARC 2003). Quantitative expression of 16 cancer related genes was measured from 0 to 15 (relative to reference genes one unit increment associated with a ~2-fold change in expression). Recurrence Score (RS) was calculated (Paik, NEJM 2004). Descriptive statistics for the RS and individual genes [ER, PR, HER2, invasion gene expression (IGE) and proliferation gene group (PGG)] among subtypes were obtained.

Results: 94.3% were ductal, lobular, or mixed. Using ductal as comparator, papillary ca had the highest ER and PR with low IGE and lowest RS. Medullary-like had the lowest ER, negative PR, higher PGG and highest RS. On average RS was lower for the classic lobular, mixed ductal/lobular, solid/alveolar lobular, tubular, cribriform, mucinous and papillary subtypes. Special histologic subtypes (tubular, cribriform, mucinous) had higher PR, lower IGE, lower PGG and higher ER (except for tubular carcinoma). Tubular cancers rarely had high RS (1.2% in high risk group), due to lower PGG and IGE.

Conclusions: Histologic subtypes have differential expression profiles. The special subtypes have higher ER, PR and lower PG and invasion IGE but outlier cases are not infrequent within this large observational cohort. The variation in gene expression by histologic subtype will be presented in detail.

Subtype	% of cases	RS (mean)	ER (mean)	PR (mean)	HER2 (mean)	Proliferation Grp (mean)	Invasion grp (mean)
Ductal	82.8	20.0	9.8	7.2	9.1	5.4	7.0
Lobular, Classic	8.0	17.5*	9.6*	7.1*	9.2*	5.1*	6.5*
Mixed	2.9	18.1*	9.8	7.2	9.2	5.2*	6.8*
Lobular, Solid/Alveolar	0.7	17.9*	10.5*	7.1	9.3*	5.7*	6.8*
Pleomorphic Lobular	0.6	18.8*	9..6*	6.9*	9.3*	5.3*	6.6*
Tubular	0.8	15.6*	9.4*	7.4*	9.3*	4.4*	6.6*
Cribriform	0.2	14.4*	10.3*	7.9*	9.4*	5.2*	6.6*
Mucinous	3.0	16.7*	10.1*	7.2	8.6*	5.3*	6.4*
Micropapillary	0.2	20.2	10.3*	7.2	9.4*	5.9*	7.1
Medullary-like	0.4	37.6*	7.9*	5.0*	8.3*	6.4*	6.4*
Papillary	0.4	12.8*	10.9*	8.1*	9.2*	5.7*	6.5*

*Significantly different compared to ductal ($p<0.05$)

151 Utility and Accuracy of Frozen Sections in Nipple Sparing Mastectomies

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Background: Nipple sparing mastectomies NSM are being performed with greater frequency in patients undergoing prophylactic and therapeutic mastectomies because of superior cosmesis. In some institutions, a subareolar margin SAM is submitted for frozen section (FS); if cancer is identified, the nipple-areolar complex (NAC) is removed in the same procedure. Existing literature on the technique has focused primarily on patient selection criteria and prospective outcomes; however, there have been no comprehensive studies examining the utility and accuracy of FS in the management of these patients. Herein we report our experience with evaluating SAM by frozen section in NSM.

Design: We reviewed all NSMs performed at our institution from 12/2007 to 8/2009. Indications for surgery were tabulated. FS diagnosis was compared to findings on permanent section of the subareolar margin. Clinical and pathological characteristics (tumor type, size and multifocality) of patients with positive and negative subareolar margins were compared.

Results: 104 NSMs from 54 patients were evaluated. FS evaluation was performed in 74 prophylactic NSMs from 38 patients with a family history of breast cancer or known BRCA mutation. 30 NSMs (18 therapeutic / 12 prophylactic) were performed on 16 patients with current or previous history of breast cancer. Frozen and permanent sections showed 100% concordance in all cases. Frozen and permanent sections of the SAM were positive in 5 of 18 therapeutic mastectomies and in 0 of 86 prophylactic mastectomies. 4 of 5 NSMs with positive SAM had multifocal disease whereas 2 of 13 NSMs with negative SAM had multifocal disease. ILC was present in 3 of 5 positive SAM. Tumor size was not statistically significant. The pathological characteristics of therapeutic NSM groups are shown in TABLE 1.

Table 1

	Positive SAM	Negative SAM
DCIS	1	6
DCIS/IDC	1	4
IDC		3
ILC	1	
Mixed IDC/ILC	2	

Conclusions: FS evaluation of the SAM can be performed accurately. FS evaluation of the SAM in prophylactic mastectomies may be unnecessary. Patients with known disease are at a higher risk of NAC involvement (fisher exact test, $p < 0.001$). Of the parameters evaluated, multifocality and ILC had a higher rate of positive SAM ($p = 0.02$ and $p = 0.01$, respectively). Patients undergoing therapeutic NSM, especially with multifocal disease, should be considered for frozen evaluation of the SAM to avoid a second procedure.

152 Withdrawn

153 Pseudoangiomatous Stromal Hyperplasia of the Breast: A Clinico-Pathological Study of 79 Cases

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Background: Pseudoangiomatous stromal hyperplasia (PASH) of the breast is a rare benign lesion that can present as a palpable nodule or as an incidental finding in breast biopsies. Microscopically it shows open slit-like spaces lined by spindle shaped cells and a dense collagenous stroma. Currently the management of patients with a core biopsy diagnosis of PASH is not well defined.

Design: This study comprised of 79 cases of PASH diagnosed at Loyola University Chicago Medical Center from 2002-2009. Clinical findings and follow-up status were collected from electronic medical records. Macroscopic data was obtained from pathology reports. The microscopic slides were reviewed to document the distribution (focal, multifocal and diffuse), type (classical and proliferative) and its association with preneoplastic and neoplastic epithelial lesions. Z-test for independent proportions is used for statistical analysis.

Results: The patients' age ranged from 15 to 65 years (mean 43 years). 76 patients were female and 3 were male. Racial status was as follows: 75.6% Caucasian, 10.5% African-American, 7% Hispanic and 6.9% others. In female cohort, 75% of patients were premenopausal, 20.2 % postmenopausal and in 4.8% the status was unknown. Ten out of 76 female patients (13.1%) were on exogenous hormone therapy. Clinically all 3 male patients had gynecomastia. 59.8% of patients presented with a breast mass

and in 40.2% the lesion was an incidental finding. Microscopically, the lesion was unifocal in 82%, multifocal in 12%, and diffuse in 6%. Classical PASH morphology was seen in 97.6% and proliferative PASH in 2.4%. Associated epithelial lesions were benign proliferative changes in 60.4%, atypical ductal and atypical lobular hyperplasia in 25.6% and infiltrating carcinoma in 14% of cases. On follow-up local recurrence was noted in 4 cases (5.2%). The finding of carcinoma in 12 out of 79 cases of PASH is statistically significant (p<0.001).

Conclusions: The finding of infiltrating carcinoma in 12 out of 79 cases of PASH is statistically significant (p<0.001). Local recurrence rate for PASH in this analysis is 5.2%. Based on these results, we recommend (1) excision biopsy for patients with a core biopsy diagnosis of PASH, (2) extensive sampling of biopsy specimen to search for significant associated lesions, and (3) appropriate clinical follow-up. Further studies would be helpful to confirm our findings and to support our recommendation for patients with PASH.

154 SIAH as a Potential Prognostic Marker for Recurrence in Patients with Ductal Carcinoma In Situ (DCIS)

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Background: Seven in absentia homolog (SIAH) is an E3 ubiquitin ligase that acts as a common downstream mediator of EGFR/Her2 and Ras signaling. The exact mechanism of SIAH in tumorgenesis is unknown, however, increased SIAH expression is seen in both neoplastic and non-neoplastic proliferating cells. Studies using shRNA and dominant negative SIAH have shown that decreased SIAH function results in decreased lung and pancreatic tumor growth *in vitro* and *in vivo*. There has been no prior examination of SIAH protein expression in breast carcinoma.

Design: Formalin fixed, paraffin embedded resection specimens from sixty-five patients with DCIS were stained with an anti-SIAH antibody (24E6H3, 1:100 dilution), and the percentage of tumor cells and normal adjacent tissue positive for SIAH nuclear staining was recorded. All patients were treated with wide excision only. Statistical analysis was performed comparing SIAH staining in tumor cells to disease recurrence, histologic type (comedo vs. non-comedo), necrosis, hormone receptor status (estrogen receptor and progesterone receptor), and Her2/neu status (Wilcoxon two-sample test), as well as nuclear grade (Kruskal-Wallis test). Correlation of SIAH expression in tumor cells with SIAH expression in normal adjacent tissue and age (Spearman correlation coefficient) was also examined.

Results: Expression level of SIAH in tumors cells was significantly higher in specimens from patients with recurrence (median=19 %) as compared to patients without recurrence (7%)(p<0.001). There was also significantly increased SIAH expression in comedo type DCIS (13.5% in comedo DCIS vs. 7% in other histologic types, p=0.014). No significant association was observed between SIAH expression and estrogen receptor, progesterone receptor, and Her2/neu status. There was a significant correlation between SIAH expression in tumors and normal adjacent tissue (Spearman correlation = 0.58, p<0.001) as well as between SIAH expression in normal adjacent tissue and patient age (Spearman correlation = -0.59, p <0.001). No significant correlation was identified between patient age and SIAH expression in tumors.

Conclusions: SIAH is a promising biomarker for disease recurrence in patients with DCIS.

155 Breast Cancer in African Women

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Background: The incidence of breast carcinoma is increasing in the African countries. It is presumed that the cancer is more aggressive and occurs in younger age, similar to that seen in the African –American (AA) women in US.

Design: I retrospectively studied twenty-one consecutive breast carcinoma specimens received in Komofo Anokye Teaching Hospital (KATH) in Kumasi, Ghana during a two-month period and compared the clinicopathological and immunohistochemical features with Caucasian (White) and African American (AA) women as described in the literature. Formalin fixed, paraffin embedded tumor tissue werestained by routine H&E and immunoperoxidase stains for ER, PR, HER-2, EGFR and CK 5/6 with appropriate controls.

Results: I found approximately 35.2% (24 out of 68) of the presenting palpable breast masses to be malignant. There is a preponderance of the usual ductal type (23 out of 24, 95.8%), mostly (90%) showing high grade (2 and 3). 55% of patients had axillary metastasis, 33% showed skin involvement and 16% had chest wall invasion. The mean tumor size was 4.6 cm and the mean age of patients was 42 years (median age 47.5 yrs). Immunohistochemical profiles of 8 cases showed majority (5 out of 8) with triple (ER, PR, HER-2) negative pattern, but did not show the usual basal phenotype. Basal cytokeratin 5/6 was negative in 7 out of 8 cases and both EGFR and HER-2 were negative in all cases.

Conclusions: In summary, the carcinomas in African women occurred in younger age, and were of higher grade and stage. Majority were triple negative, ductal type but not of typical luminal or basal cell types. These findings are different from the Caucasian Americans but somewhat similar to the African Americans and may have prognostic implications in the breast cancer patients of sub-Saharan Africa.

156 Semi-Quantitative Estrogen Receptor Expression Level Influences Response to Trastuzumab Containing Neo-Adjuvant Chemotherapy in HER2 Positive Tumors

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Background: Prior to 2006, use of trastuzumab in neoadjuvant chemotherapy (NACT) regimen was limited to clinical trials. Recently, trastuzumab is increasingly used pre-operatively in HER2+ breast carcinoma, often as TCH regimen (Taxotere, Carboplatinum, Herceptin). Pathologic complete response (pCR) to NACT without trastuzumab in hormone receptor negative/HER2+ tumors is seen in 27% to 45% cases. In contrast, estrogen receptor (ER)+/HER2+ tumors demonstrate pCR in ~8% cases and is generally limited to weak to moderate ER+/HER2+ tumors (Cancer, in press). It is speculated that addition of trastuzumab to NACT regimen will increase the pCR rates in all HER2+ tumors.

Design: A list of HER2+ patients that received TCH NACT in the years 2006-2007 was obtained from our hospital tumor registry. The 34 HER2+ tumors were classified in 3 groups based on semi-quantitative hormone receptor and HER2 results as follows: ERBB2, Luminal A-HER2 Hybrid (LAHH), Luminal B-HER2 Hybrid (LBHH). The criteria for classification are shown in the table below. pCR was defined as absence of invasive carcinoma in the resection specimen and in the lymph nodes. Percentage tumor size reduction was also calculated based on pre-therapy size and detailed evaluation of the resection specimen.

Results:

pCR in various categories of HER2+ tumors

Tumor Class	Immunohistochemical Criteria	pCR	Average Tumor Size Reduction
ERBB2	ER/PR negative, HER2+	12/18 (67%)	88%
LAHH	Strong ER+, HER2+	2/11 (18%)	66%
LBHH	Weak/moderate ER+, HER2+	2/5 (40%)	90%

pCR: Pathologic Complete Response

The difference in pCR rates among 3 categories was borderline significant (p value: 0.059). Average tumor size reduction was significantly higher in ERBB2 and LBHH tumors compared to LAHH tumors (p value: 0.003).

Conclusions: Addition of trastuzumab to NACT regimen significantly increases the pCR rates in all HER2+ tumors. However, the benefit of trastuzumab is highest in ER negative tumors and progressively decreases with increase in tumor ER expression. This information can be utilized to counsel patients considered for NACT and the same principle could be applied in the adjuvant setting. Generally patient that achieve pCR have excellent prognosis compared to the one that fails to achieve pCR. Whether addition of trastuzumab in NACT regimen would have the same effect will be analyzed in the years to come.

157 Significance of Apocrine Lesions in Breast Core Biopsies. *A* Comprehensive Retrospective Review of Seven Years

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Background: Apocrine differentiation is seen within a wide range of lesions of the breast. Although apocrine metaplasia is one of the most common findings in breast core needle biopsies (CNB), little is known about the significance of apocrine change and the risk of and association with subsequent carcinoma. The aim of this study is to classify breast conditions with apocrine change and evaluate the prognostic significance of apocrine lesions in CNB.

Design: A computer data search review of our pathology records was performed for patients who underwent CNB and reported with "apocrine" between January 2002 and September 2009. Follow-up resection reports and any subsequent excisions were reviewed as well.

Results: A total of 22,455 CNB were performed during the period of 2002-2009 at our institution. Apocrine lesions comprised **7.5%** (1686/22,455) of all CNB lesions. The mean age was 53.8 years. 93.8% (1581/1686) were found in women >40 years, 6.2% (106/1686) were in women <40years. Benign apocrine lesions (**Group-1**) were identified in **80.5%** (1357/1686), atypical apocrine proliferations (**Group-2**) in **1.6 %** (27/1686) and malignant apocrine proliferations (**Group-3**) in **17.9%** (302/1686). Histologic follow-up was available in **6%** (96/1686) cases [Group 1=50, Group 2=15, Group-3=31]. The mean follow-up period was 12 months (1 month-6 years). Follow-up excisional results are shown in Table 1.

INITIAL BIOPSY	FOLLOW-UP	
BENIGN		
Apo metaplasia (AM)	AM/PACAM	32
PACAM	AM/PACAM	7
PACAM+apocrine adenosis	PACAM+apocrine adenosis	1
AM	DCIS/Apo DCIS/IAC	10
ATYPICAL		
Atypical apocrine	Atypical apocrine/ADH/Apo DCIS	11
Atypical apocrine+ADH	DCIS+ADH/ADH	3
Atypical apocrine+DCIS	IAC	1
MALIGNANT		
Apo DCIS	Apocrine adenosis/AM/PACAM/Atypical apocrine/Apo DCIS	17
Invasive apo carcinoma (IAC)	AM/PACAM/Apo DCIS/IAC	14

In **80%** (40/50) cases of Group 1, the initial benign lesion persisted and in **20%** (10/50) cases, the apocrine lesion evolved or was associated with a malignant lesion in the follow-up excisions.

Conclusions: Our study shows that apocrine change is a common and persistent feature in breast lesions. In **Group-2**, a significant high risk lesion (DCIS or invasive apocrine carcinoma) is seen in 40% (6/15). Apocrine changes in malignant conditions are persistent. Our observations show in **20%** of **Group-1**, the apocrine lesion was associated with a malignant lesion in the follow-up excisions. The potential "precursor" role of a subset of apocrine lesions and the biologic implications for apocrine malignant lesions needs to be evaluated in future large prospective studies.

158 A Comparative Study of p16 Expression in Basal-Like Carcinoma and High-Grade Invasive Ductal Carcinoma of the Breast

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Background: Basal-like carcinomas of the breast (BLCB) are one of the molecular subtypes determined by gene expression profiling. BLCB generally have a high mitotic index, are ER-/PR-/HER2- ("triple-negative breast cancer"), and show variable

expression of CK 5/6, CK14, EGFR and CD117. $p16^{INK4a}$ expression has been identified in HPV-related carcinomas of genital tract and oropharynx. Little is known about $p16^{INK4a}$ and BLCB. The aim of this study was to determine $p16^{INK4a}$ expression in a subset of BLCB and compare the results with a group of high-grade invasive ductal carcinoma (HG-IDC) of breast.

Design: Tissue microarrays (TMA) were constructed in triplicate including 18 HG-IDC and 18 BLCB. Whole tissue sections were also used for BLCB. Immunohistochemical (IHC) stains for ER, PR, HER2, CK5/6, CK14, EGFR, CD117 and p16 were performed. The slides were stained using DAKO autostainer. Positive and negative controls were applied.

Results: The IHC results are summarized in Table 1.

		BLCB	HG-IDC
p16	Positive	16 (89%)	10 (55.5%)
	Negative	2 (11%)	8 (44.5%)
CK5/6	Positive	17 (94.4%)	5 (28%)
	Negative	1 (5.6%)	13 (72%)
CK14	Positive	17 (94.4%)	3 (17%)
	Negative	1 (5.6%)	15 (83%)
EGFR	Positive	14 (78%)	2 (11%)
	Negative	4 (22%)	16 (89%)
CD117	Positive	13 (72%)	2 (11%)
	Negative	5 (28%)	16 (89%)
ER	Positive	0 (0%)	2 (11%)
	Negative	18 (100%)	16 (89%)
PR	Positive	0 (0%)	3 (17%)
	Negative	18 (100%)	15 (83%)
HER2	Positive	0 (0%)	3 (17%)
	Negative	18 (100%)	15 (83%)

All BLCB cases were ER-/PR-/HER2-. Seventeen BLCB were CK 5/6+/CK 14+; EGFR was positive in 14/18 BLCB and CD117 was expressed in 13/18 BLCB. Nuclear diffuse expression of $p16^{INK4a}$ was seen in 16/18 (89%) BLCB, ranging from 80% to 100%; nuclear $p16^{INK4a}$ staining was present in 10/18 (55.5%) HG-IDC, ranging from 5% to 100%.

Conclusions: Our study shows that $p16^{INK4a}$ was highly expressed in the majority of BLCB (89%) in comparison to HG-IDC (55.5%). In triple-negative breast cancer cases with CK5/6 and CK14 co-expression, $p16^{INK4a}$ may serve as an additional biomarker for identification of BLCB.

159 Molecular Analysis Evaluation of Axillary Nodal Micrometastasis in Patients with Breast Cancer

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Background: The significance of micrometastasis (MiM) versus macrometastasis (MaM) in breast carcinoma (BC) impacts pathology staging and management. Currently, axillary lymph node (LN) deposits less than 2 mm are disregarded however such a finding could represent early spread of tumor. Two different mechanisms can account for this occurrence: 1) passive dislodgement of cancers cells from the primary site in breast to draining LN or 2) lymphatic invasion by aggressive tumor with active growth and dissemination. Ideally, pathology diagnosis and staging should reflect the true status of the cancer cell biology in the individual patient. To address this we performed comparative mutational profiling of primary BC and corresponding MiM and MaM LN deposits.

Design: 14 cases of breast ductal carcinoma with nodal MiM and 9 control cases consisting of breast ductal carcinoma with nodal MaM (total 23 cases) were retrieved from the hospital computer system. All cases were confirmed by histology and immunohistochemistry (IHC) and were microdissected using unstained formalin fixed, paraffin embedded standard tissue sections. Microdissection targets consisted of one non-neoplastic site, 2-3 sites of primary BC and LN metastatic deposits. Each microdissected sample was quantitatively genotyped and time course of mutation acquisition determined using a broad panel of 17 mutational markers including k-ras-2 point mutation and loss of heterozygosity (LOH) for 1p, 3p, 5q, 9p, 10q, 17p, 17q, 21q, 22q.

Results: All control cases of MaM disease showed the same number or greater cumulative mutations in LN deposits and this was used to define two states: active growth (MaM disease) versus passive spread (LN deposit showed fewer cumulative mutations than primary tumor). Of the 14 cases with MiM, 9/14 (64.3%) showed fewer mutations in the nodal MiM versus the primary BC, supporting passive displacement rather than true metastasis. 5/14 cases (35.7%) showed biologically active disease with more mutations in the nodal MiM versus the primary BC, supporting true biological metastases.

Conclusions: Our results support the need for individualizing pathologic staging of BC with respect to the biological nature of MiM disease. We believe molecular analysis is a useful technique to better evaluate the significance of MiM in axillary lymph nodes. The methods used here support its implementation in correlative studies with outcome analysis.

160 mTOR Immunohistochemical Expression Is of Prognostic Relevance in High Risk Breast Carcinoma

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Background: The PI3K/AKT/mTor pathway is of pivotal relevance in breast cancer. Its alterations may be of prognostic/predictive value. mTOR is a protein kinase of the PI3K/AKT signalling pathway and central role in controlling cancer cellular growth and cell cycle progression . The mTOR pathway is abnormally actived in many tumors and could be a target for specific therapy. Sirolimus and its analogues Temsirolimus, Everolimus, and Deforolimus are specific small molecule inhibitors of mTOR and some of these agents are now being examined as novel therapies for cancer.

Design: We evaluated a series of 165 consecutive infiltrating breast carcinomas with long term follow-up (median f-up: 85 months). The clinico-pathological characteristics of the series were: 80 T1, 63T2, 22 T3/4, 12 G1, 75 G2, 68 G3, 5 Gx, 76 N0, 85N1, 4 Nx. Cases were grouped according to St. Gallen risk categories in low (4 cases), intermediate (97 cases) and high (48 cases) risk (for 19 cases risk category was not asssable). All cases had been studied for ER, PgR, Her2, p53 and MIB1 immunohistochemical expression and for PI3K and AKT mutations (as previously shown Clin Cancer Res. 2008; 14(6):1726) All cases were included in tissue microarrays, and immunostained for PTEN and mTOR. PTEN and mTOR immunoreactivity were scored as low to absent (score 0-1) and strong (score 2) on a semiquantitative basis. Follow-up data and therapy were recorded for all cases.

Results: mTOR expression were not related to any pathological parameter nor with ER, PgR, p53 and Her2 status nor with PTEN, PI3K and AKT alterations. Survival analysis showed that high mTOR expression was related with prolonged overall and relapse free survival in several groups of patients, including node positive, chemotherapy treated patients and in high risk subgroups.

Conclusions: Our results suggest that high risk breast cancer patients who are mTOR positive might have prolonged survival and further studies are required to evaluate if mTOR expression could be of relevance in the selection of patients who could be candidates for mTOR inhibition therapy.

161 Differences in AKT Pathway Detected by Digital Quantification of Protein Expression in Different Subtypes of Breast Cancer

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Background: AKT pathway is involved in breast cancer progression and differential expression among molecular subtypes of breast cancer has been described. Some new modulators of AKT pathway are under investigation for clinical treatment and quantification at the protein level may be clinically relevant. So far no standard method to quantify the AKT pathway is available.

Design: We evaluated protein expression by immunohistochemistry of AKT, pAKT, mTOR, PTEN, ER, PR and Her2 performed using Tissue microarrays including 56 breast carcinomas, T1/T2 without metastasis in patients between 40 and 70 years old using tissue microarray. Cases where classified based on the expression of estrogen receptor and Her2. The digital quantification was performed using the ACIS III microscope. Local invasion and proliferation parameters were compared to the molecular profile and AKT, pAKT, mTOR and PTEN levels of expression.

Results: The mean age was 53 and the mean tumor size was 2.65 cm (0.4 to 4.5 cm). The mean cytoplasmic expressions were AKT=109.74, pAKT=86.21, mTOR=96.85 and PTEN=121.01. Nuclear staining was observed in 18 (36.0%), 23 (46%), 0 (0%) and 29 (56.7%) cases of AKT, pAKT, mTOR and PTEN, respectively. Triple negative tumors were associated with high mitotic index (p=0.036), low cytoplasmic expressions of AKT and mTOR (p=0.027 and p=0.037, respectively). There was no association between AKT, pAKT, mTOR or PTEN expression and tumor size, number of mitosis or mitotic index. We observed a positive and significant correlation between the expression of PTEN and AKT (r = 0.40; p = 0,003); PTEN and pAKT (r = 0.32; p = 0.012); but no correlation between PTEN and mTOR (r = 0.06; p = 0.76).

Conclusions: Triple Negative tumors showed lower cytoplasmic expression of AKT and mTOR compared to Luminal A tumors. The high mitotic index observed in triple negative tumors was not associated to AKT pathway overexpression. The digital quantification using immunohistochemistry can detect differences in protein expression of AKT pathway among breast cancer molecular subtypes and may be of clinical use.

162 Mitotic Grade and MIB-1 Are Not Redundant Measures of Proliferation in Breast Cancer

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Background: In breast cancer, the proliferation rate measured by MIB-1 immunohistochemistry allows sub-typing of estrogen receptor positive breast cancers that closely approximates genetic-based tests and predicts response to therapy. Whether MIB-1 data complements or replaces traditional light-microscopic assessments of mitotic grade (MG) is not established. We evaluated the interaction between MIB-1 and MG using disease-free survival as the outcome variable.

Design: Tumor samples were available for 1329 patients (89%) randomized to the Breast Cancer International Research Group 001 phase III trial comparing docetaxel, doxorubicin and cyclophosphamide (TAC) with fluorouracil, doxorubicin, and cyclophosphamide (FAC) in women with operable, node-positive breast cancer. Disease-free survival was assessed at a median follow-up of 55 months. Both the MIB-1 index and MG were assessed by a single observer blinded to treatment and outcome.

Results: Tumors were classified MG 1, 2, or 3 (52%, 25%, and 22%, respectively). The distribution of MIB1 scores was mathematically modeled for two normal populations and the best discriminatory cut-point of greater than or equal to 30% positively staining cells was adopted. This defined MIB-1Lo (49%) and MIB-1Hi (51%) tumors. Both MG and MIB-1 were significant prognostic indicators (univariate analysis) and highly correlated (Spearman correlation coefficient, $p<0.0001$). 59% of cases were perfectly concordant as MG1 and MIB-1Lo (n=507) or MG3 and MIB-1Hi (n=276). Only 2% (n=22) were MG3 and MIB-1Lo and excluded from further analysis. Using the most concordant group (MG3, MIB-1Hi) as the basis for comparison, there was a statistically significant interaction between MG and MIB-1. MIB-1Lo and MIB-1Hi defined significantly different prognostic groups within the MG1 and MG2 tumors (p=0.0004 and p=0.007 respectively). Similarly, MG divided the MIB-1Hi population into different prognostic groups (p=0.006). In a pairwise comparison, patients with MG3 tumors had a relative risk of relapse of 1.68 compared with MG1 (p=0.0019).

Conclusions: MG is not redundant with MIB-1 and gives complementary prognostic information. There is a possibility that the two markers may be integrated to provide cell cycle information that predicts chemotherapy response.

163 Androgen Receptor Expression Is a Significant Prognostic Factor in Estrogen Receptor Positive Breast Cancers Treated with Chemo-Endocrine Therapy

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Background: Controversies remain regarding the role of androgen receptor (AR) in breast cancer patients. This study was to evaluate the prognostic value of AR expression in patients with estrogen receptor (ER) positive breast cancer treated with endocrine therapy with or without the addition of chemotherapy.

Design: A consecutive series of 953 patients with ER-positive breast cancer treated between 1998 and 2003 was selected. Repeated immunohistochemistry confirmed the expression of ER in the tumor of 938 patients. AR expression was measured by immunohistochemistry. The Kaplan-Meier method, logrank test and multivariate Cox models were used to explore the impact of AR expression on time to relapse (TTR) and disease specific survival (DSS) in all patients and in subgroups treated with chemo-endocrine therapy or endocrine therapy alone.

Results: AR immunoreactivity was assessable in 859 tumors and positive in 609 (70.9%). In the group of patients receiving chemotherapy AR positivity was a strong prognostic factor for TTR (P=.015) and DSS (P<.001). In this subset Cox models showed that AR was an independent variable for both TTR (P=.003, HR 0.444, 95%CI 0.258-0.765) and DSS (P<.001**,** HR 0.135, 95%CI 0.054-0.337). Finally, among the patients with tumors showing immunophenotypical features of luminal B breast cancer, AR expression identified a group of patients with better prognosis for TTR (P=.017, HR 0.521, 95%CI 0.306- 0.888) and DSS (P=.001**,** HR 0.276, 95% CI 0.130- 0.588).

Conclusions: AR expression is an independent prognostic factor of better outcome in patients with ER-positive breast cancers.

164 Micropapillary Ductal *In Situ* Carcinoma of the Breast: An Inter-Institutional Study

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Background: The clinical significance of micropapillary growth pattern in ductal *in situ* carcinoma is controversial and the impact of nuclear grading in terms of recurrence of this lesion is yet to be clarified. Our aim was to evaluate, on a series of micropapillary in situ carcinomas, the histological features correlated with recurrence and whether the micropapillary subtype had a different behavior from other non--micropapillary ductal carcinoma *in situ*.

Design: We collected 55 cases of micropapillary *in situ* carcinomas from four institutions. All cases were reviewed for nuclear grade, extent, necrosis, microinvasion and tested for estrogen and progesterone receptors, Ki67, HER2, EGFR and p53 expression. Clinical data, type of surgery and follow up were obtained for all patients.

Results: Our results showed that the nuclear grade is crucial in determining the biology of micropapillary in situ cancer, so that the high nuclear grade micropapillary ductal carcinoma *in situ* more frequently overexpressed HER2, showed higher proliferation index, displayed necrosis and microinvasion and was more extensive than low/intermediate nuclear grade micropapillary ductal carcinoma *in situ* . Logistic regression analysis confirmed the high nuclear grade (Odd Ratio: 6.86; CI: 1.40–33.57) as the only parameter associated with elevated risk of local recurrence after breast conserving surgery. However, the recurrence rate of 19 micropapillary *in situ* carcinoma, which were part of a cohort of 338 consecutive ductal carcinoma *in situ*, was significantly higher (log-rank test, *p-value* = 0.019) than that of non-micropapillary, independently of the nuclear grade.

Conclusions: Although nuclear grade may significantly influence the biological behavior of micropapillary ductal carcinoma *in situ*, micropapillary growth pattern *per se* represents a risk factor for local recurrence after breast conserving surgery.

165 Correlation of CCND1 Amplification with Hormone Receptor (ER, PR) Expression and ERBB2 (Her2/neu) Gene Amplification in Invasive Ductal Carcinoma of the Breast: A Study of 102 Cases

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Background: The cyclin D1 gene (CCND1), located on human chromosome 11, is an important oncogene and is considered a major driver in breast carcinogenesis. Cyclin D1 is a downstream target of hormone receptors (ER, PR) and Her2/neu (ERBB2), important therapeutic markers in breast cancer classification. High levels of Cyclin D1 are associated with poor prognosis and therapeutic resistance in hormone-receptor positive cancers. However, the prevalence and significance of this abnormality in "Her2 positive" and "triple negative" breast cancers is poorly described. The purpose of this study is to evaluate CCND1 amplification in breast carcinoma by fluorescence in-situ hybridization (FISH), and to correlate these findings to ER, PR, and ERBB2 status in invasive ductal carcinomas of the breast.

Design: Consecutive cases of invasive ductal carcinoma of the breast were identified, tumor tissue was formalin-fixed and paraffin-embedded, tissue cores were obtained from multiple foci of each tumor, and tissue microarrays were prepared. Probes binding to either CCND1 (11q13) or ERBB2 (17q21-q22) and, respectively, chr 11or17 centromeric probes were used in FISH analysis of tissue microarray sections, with satisfactory signals achieved in 102 cases. Tumor cores were scored for CCND1 and ERBB2 amplification, defined as a signal ratio of 2.0 or greater. Results were correlated with ER and PR status determined by immunohistochemistry on whole tissue sections.

Results: Among 102 cases of invasive ductal carcinoma, 56 were ER+/Her2-, 15 were Her2+, and 31 were triple-negative. Overall, 12/102 demonstrated CCND1 amplification by FISH (12%). CCND1 amplification was seen in 12.5% (7/56) of ER positive/Her2 negative cases, 20% (3/15) of Her2 amplified cases and only 6% (2/31) of "triple negative" cases.

Conclusions: Findings demonstrate that CCND1 amplification is seen in invasive ductal carcinomas of all major subtypes, including ERBB2 amplified and triple negative breast cancers. Differences in the prevalence of CCND1 amplification among the subtypes did not achieve statistical significance in this study. Additional study of CCND1 amplification and its potential prognostic and therapeutic relevance in these less common subtypes is warranted.

166 Lobular Carcinoma Progression Model: Immunohistochemical Analysis on Tissue Microarray

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Background: Lobular carcinoma in situ (LCIS) is generally considered a marker for higher risk. However, there is evidence to show the progression of LCIS to invasive lobular carcinoma (ILC) as the two share some similar genomic alterations including chromosome 16q loss and 1q gain. In this pilot study, our objective was to seek the role of several carcinogenic markers in the evolution and progression of the lobular carcinoma of the breast.

Design: We generated a tissue microarray (TMA) including cores from normal lobules, LCIS and ILC in each case. Immunohistochemical (IHC) antibodies for E-cadherin, β-catenin, IGF1, IGF2, EGFR, ER, PR, her2, BRCA1, BRCA2, MLH1, MSH2, p53, bcl-2 and cyclin D1 were applied to the sections made from the TMA paraffin blocks. We scored each of the three components (i.e. normal lobule, LCIS, ILC) in every core separately by adding the score for distribution (0-4) to the score for intensity (0-3) with a maximum score of 7. Scoring of her2 was performed according to the Dako scoring system.

Results: We randomly identified 39 cases diagnosed as ILC (mean age: 62.4 years). In all cases, the LCIS and ILC were completely negative for E-cadherin. Membranous β-catenin staining was observed in 79% of normal lobules, 12.8% of LCIS and 20.5% of ILCs. Cytoplasmic (Golgi pattern) β-catenin staining was observed in 0% of normal lobules, 18% of LCIS and 38% of ILCs. See table 1 for IHC analysis.

Immunohistochemical Scores

	Normal Lobules (mean)	LCIS (mean)	ILC (mean)
IGF1	4.6	5.2	5.4
IGF2	5.0	5.5	5.6
EGFR	0.4	0	0
BRCA1	5.2	5.4	5.4
BRCA2	2.9	1.8	2.4
MLH1	3.2	3.9	3.8
MSH2	5.4	5.3	5.8
p53	0	0	0.3
cyclin D1	0	0.2	0.2
ER	2.2	3.7	3.6
PR	1.7	2.2	2.5
her2	0	0.1	0.1
bcl2	3.7	4.0	4.2

Maximum score=7; her2 score: 0-3

See table 2 for the clinicopathologic data.

Clinicopathologic Data

Laterality	Left	Right		
	21 (53%)	18 (47%)		
Subtype	Classical ILC	Pleomorphic ILC		
	34 (87%)	5 (13%)		
pT	T1	T2	T3	
	20 (51%)	16 (41%)	3 (8%)	
pN	N0	N1	N2	N3
	21 (53%)	12 (31%)	3 (8%)	3 (8%)
LVI	Present	Absent		
	8 (20%)	31 (80%)		

ILC: Invasive lobular carcinoma; LVI: Lymphovascular invasion

Conclusions: BRCA1, BRCA2, MLH1 and MSH2 are intact in lobular carcinoma. No diffuse overexpression of p53, EGFR, her2 or cyclin D1 is noted in lobular carcinoma. ER and PR are more frequently expressed in LCIS and ILC than normal lobules. IGF1, IGF2 and bcl-2 show slight incremental values from normal lobules to ILC. Incremental abnormal β-catenin staining was observed in LCIS and ILC.

167 Downregulation of Hematopoietic Progenitor Kinase 1 Protein Level in Ductal Carcinoma In Situ and Invasive Ductal Carcinoma of Breast

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Background: Hematopoietic progenitor kinase 1 (HPK1) regulates stress response, proliferation, and apoptosis in hematopoietic cells. The role of HPK1 is not well studied in solid tumors. Recent study in our lab showed that the loss of HPK1 is associated with the progression of pancreatic intraepithelial neoplasm and invasive pancreatic ductal

adenocarcinoma, and it may contribute to the tumorigenesis of pancreatic cancer. In this study, we will examine the HPK1 protein expression during the development of invasive ductal carcinoma of the breast.
Design: Forty three cases of normal breast tissue, thirty two cases of ductal carcinoma in situ (DCIS), and forty nine cases of invasive ductal carcinoma (IDC) were collected from formalin-fixed, paraffin-embedded archival tissue blocks. HPK1 protein level was evaluated by immunohistochemical staining. Tumors or benign breast tissue with no or only focal cytoplasmic staining for HPK1 (<5% of the cells) was regarded as negative. Tumors or benign breast tissue with cytoplasmic staining for HPK1 (≥5% of the cells) was regarded as positive.
Results: 29/43 normal breast tissue (67.4%) show positive staining of HPK1 protein in the normal ductal epithelial cells. In contrast, only 6/32 DCIS (18.7%) show positive staining (P<0.0001) and 8/49 IDC (16.3%) show positive staining (P<0.0001). HPK1 is lost in DCIS and IDC, particularly in those with higher grade lesion.
Conclusions: We demonstrated that HPK1 protein is lost in a high percentage of DCIS and IDC compared to the normal ductal epithelial cells. These results suggest that loss of HPK1 protein may play a role in the oncogenesis of the breast cancer.

168 Sentinel Lymph Node Biopsy (SLNB) in Ductal Carcinoma In-Situ (DCIS) – A Study of 90 Cases

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Background: Sentinel lymph node biopsy (SLNB) is well established for invasive ductal carcinoma but the role of this procedure in DCIS remains controversial. Most breast cancer management guidelines discourage axillary lymph node staging in pure DCIS when breast conserving surgery (BCS) is performed. SLNB for DCIS is currently limited to patients who undergo mastectomy. We sought to review our experience of SLNB in DCIS, and to determine from our database whether SLNB is appropriate in all patients with DCIS electing mastectomy.
Design: From April 1995 through December 2008, 90 cases of DCIS in whom SLNB were performed. SLNB in DCIS was done if elected or at surgeon's discretion. All the SLNs were step-sectioned and entirely submitted. Immunohistochemistry for cytokeratin was not performed routinely.
Results: 77/90 cases were pure DCIS, 13/90 DCIS with microinvasion (DCISM). 70/90 patients had a mastectomy and SLNB (68-DCIS, 2-DCISM). BCS with SLNB was done in 20/90 (9-DCIS, 11-DCISM) for extensive DCIS. 5/90 (4-DCIS, 1-DCISM) (5.6%) revealed metastatic tumor in the SLN (+SLNB) 4 of which had mastectomy. 3 patients underwent complete axillary lymph node dissection; none had metastasis in non-SLN. 4/5 +SLNB cases had tumor size ≥ 4 cm[4 cm-DCISM, 4.5 cm, 4.6 cm,11.0 cm-DCIS; mean:6.03 cm, standard deviation:3.33] and all high nuclear grade with necrosis. The 5th +SLNB had 1cm DCIS size but had contralateral invasive ductal carcinoma with metastatic SLN. Among 32 cases of DCIS with size ≥ 4.0cm; 4 (12.5%) had +SLNB and 18/32 (56.3%) were high grade.
Conclusions: Metastasis to SLNs occurred in 5.6% of patients with DCIS. The incidence of SLN metastasis increased substantially in those with DCIS > 4 cm; in these patients microinvasion was identified in about 20%. Non-sentinel node metastasis did not occur. Patients who have a mastectomy for a large DCIS should have a SLNB since microinvasion, and therefore potential SLN metastasis, is related to increasing size. If SLNB is not done in these mastectomy patients, and if microinvasion is found on histology, the only surgical option is complete axillary lymphadenectomy.

169 Expression of Cyclin D1, MIB-1 (Ki-67) and Estrogen Receptor (ER) in Flat Epithelial Atypia (FEA)

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Background: The clinical significance of FEA in the literature is beginning to emerge; recent studies (*kunju et al, chivukula et al*) have shown a significant upstaging in FEA up to 14%. Though very few papers have defined FEA, at times as having "bland nuclear cytology" sometimes difficult to differentiate these lesions from benign CCC. In the pursuit to find useful markers that can recognize these lesions from benign CCC, we have selected three markers. Literature on Cyclin D1, a cell cycle regulatory protein, is meagre in relation to breast carcinoma, although alterations were seen in apocrine metaplasia (APM).This protein is seen in various lymphomas suggesting cellular instability contributing to increased susceptibility to carcinogenesis. The aim of the study is to study the expression of Cyclin D1, MIB-1(ki-67) and ER in FEA and CCC.
Design: Twenty (20) cases of pure FEA cases were selected. We defined FEA as per the WHO criteria. Positive cyclin D1 expression is defined as strong nuclear staining in >20% of cells (*colombo et al*). A proliferation index (PI) is calculated for MIB-1(ki67) a nuclear stain as low- <10%, moderate 11-25%, high 26-50%, and high>50%. For ER, a 10% or more "nuclear "staining of the tumor cells was considered "positive"A cumulative "H score" is derived based on proportionality (PS) and intensity scores (IS) [0 negative, 1-150 low, 151-250 intermediate, 250-300 high].
Results: A strong ER expression was seen in 100% (18/18) FEA cases with a mean H score of 195 (range 140-220); a low MIB -1 (ki-67) staining was seen in all cases with a mean proliferation index of 3.2 A strong intense nuclear staining of Cyclin D 1 was seen in up to 95% (19/20) of cases. In the adjacent CCC, a strong ER expression was seen in 100% (18/18) of cases. However there was overtly less cyclin D1 staining in 100% (20/20) of cases. MIB-1 (ki-67) was absent /weak staining with a mean proliferation index of 1.0.
Conclusions: 1. FEA are proliferating lesions as demonstrated by increased MIB-(ki-67) in comparison to CCC. **2.** Increased expression of Cyclin D1 in FEA suggests alterations that may contribute to the role of these lesions in the low grade breast cancer pathway. **3**. A high expression of ER in these lesions suggests their possible "precursor" role in the predisposition of women to develop invasive breast carcinoma.**4.** A combination of these markers can be used to distinguish these lesions from benign CCC.

170 Cytoplasmic Expression of Estrogen Receptors in Breast Cancer

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Background: Nuclear expression of estrogen receptor (ER)α has been used as the prognostic and therapeutic marker for breast cancer. However, ER cytoplasmic expression (CyE) has not gained such support of its contirbution and clinical significance. Membrane ER or unliganded cytoplasmic hormone receptors may represent non-genomic actions of ER or subcellular dislocation during malignant transformation due to inappropriate /dysfunctional trafficking of transcription factors and cofactors. Here, we tested CyE of ERs in breast cancer tissues and breast cancer cell lines (BCC) to further define its role.
Design: Tissue microarray sections of 271 cancers, 45 normals, 62 benign proliferative disease (BPD) of breast tissues and 11 BCC (MCF-7, T-47D, ZR-75, MB-361,MB 468, BT 474, MB-453, SK-BR-3,MB-231, Hs578T, BT-549) were tested by immunohistochemistry for the expression of ERα (ID5, Dako), 5 different clones of ERβ (AR385-5R, Biogenex; PPG5/10, Dako;Ab288/14C8, ABCAM; EMR02, Novacastra; 57/3, AbD Serotec), Her-2 (HercepTest, Dako), Ki-67, AIB-1 (Clone 34, BD), TIF-2 (Clone 29, BD). Non-specific protein was blocked by using serum block (Dako, CA). Over 5% positive nuclear and cytoplasmic expression in each tissue core was considered positive.
Results: ERα-CyE was noted in 9.0% (27/271) of breast cancers; 6 were ERα-/Her-2+, and 3 were ERα+/Her-2+. One BCC, BT474 with ERα+/Her-2+ showed ERα-CyE. ERβ-CyE was noted in 29.1 % (81/271) of cancers and 4 of 11 BCC. CyE of 5 ERβ clones were consistent expression in each breast tissue and BCC. The staining of ERβ-CyE in BCC showed diffuse, membranous or dotted appearance. In breast cancer, that of ERβ-CyE was diffuse and was prominent in high grade cancer with desmoplastic reaction and heavy lymphocytic infiltration. ERβ-CyE was noted in 47.0 % (47/100) of stage IV cancer including recurrent and tamoxifen resistant cancers, and 19.2 0% (33/171) stage I to III cancers. ERβ-CyE was associated with over expression of Her-2 in 22.2% (18/81), Ki-67 in 23.4% (19/81), TIF-2 in 46.5% (38/81) and AIB-1 in 21.0% (17/81). Only cytoplasmic ERα and ERβ in breast cancer was noted in 3.3% (9/271) and in 8.4% (23/271), respectively. CyE levels in normals and BPD tissues were insignificant.
Conclusions: Our study demonstrates the potential significance of cytoplasmic ER, particularly ERβ. Proteins translated from single and multiple exon deletion variant transcripts which lack nuclear localization signals may remain in the cytoplasm of breast cancer tissues and contribute to estrogen responses. Further study is in progress to test cytoplasmic expression of various ER isoforms.

171 Relevance of Multiple Breast Cancer Cell Lines as Models for Breast Cancers

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Background: Back ground: Breast cancer consists of a heterogenous population of cancer cells. Testing multiple breast cancer cell lines (BCC) would reflect the reasonably acceptable model of breast cancer in vivo. Most studies involve only a few BCC. Here, we tested 11 different pheno and geno-types of BCC focusing on ERβ expression together with breast cancer tissues as previous studies on ERβ have shown inconsistent results.
Design: BCC consisted of (1) MCF-7, T-47D, ZR-75 representing luminal epithelial-like cell type, (2) MDA-MB-361,MB 468, BT 474, MDA-MB-453, SK-BR-3 as weakly luminal epithelial-like type with Her-2 over expression, and (3) MDA-MB-231, Hs578T, BT-549 highly invasive, stromal/mesenchymal-like type. Tissue microarray sections of 11 BCC and 224 breast cancer tissues were tested for the expression of ERβ isoforms using three different clones (PG10, Dako;14C8,ABCAM ;AR385-5R, Biogenex) and also for ERα (ID5, Dako), Her-2 (HercepTest, Dako), PR, smooth muscle actin, vimentin, cyokeratin (AE1/AE3), and CK5/6 using the standard immunohistochemistry procedure after appropriate antigen retrieval and serum blocking. Over 5% nuclear or cytoplasmic expression was considered to be positive.
Results: Nine BCC except BT 549 and Hs578T maintained epithelial phenotype with positive cytokeratin. The luminal type, MCF-7 and T47D, ZR-75 were ERα+/PR+/Her-2-/cytokeratin + /vimentin- with high expression of ERβ. Five weakly luminal types were ERα-/PR+/Her-2 + /vimentin-/actin-/ERβ+ and 2 were ERα-/PR-/Her-2-/ERβ+/ vimentin-/actin-. The stromal /mesenchymal type was ERα-/PR-/Her-2-/vimentin+/-/ actin+/- with low ERβ expression. CK5/6 stain was seen only in MB 468. ERα and ERβ were co-expressed mostly in the luminal types. ERα negative and triple negative BCC showed low ERβ expression. In breast cancer tissues, ERβ was expressed in 55.4% (124/224) in all, and in 25 % (28/112) of ERα negative and 49% (29/61) of triple negative breast cancer tissues.
Conclusions: BCC lines with different pheno- and geno-types may reflect reasonably acceptable models to evaluate breast cancer cells *in vivo.* ERβ expression in BCC and breast cancer tissues were comparable. More specifically, BT-549, Hs578T, MDA-MB-231 can represent the mesenchymal type, 'epithelial–mesenchymal transition' type and MB 468 the basal-like type. Study are in progress to test RNA expression of ERβ isoforms with different agonist and antagonists to explore the targeted therapeutic potential of ERβ in ERα negative and Triple negative breast cancers.

172 ERβ, AIB-1, and TIF-2 Expression in Breast Cancer-Associated Myofibroblasts

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Background: Myofibroblasts (MF) are the predominant cells in cancer microenvironment orchestrating the epithelial-mesenchymal crosstalk. MF may play a role through genomic or non-genomic pathways in cancer progression. Two steroid receptor coactivators, amplified in breast cancer-1 (AIB-1) and transcriptional intermediary factor 2 (TIF-2) are enhanced in epithelial cells of various cancer but their role in MF in breast cancer

remains elusive. We investigated the expression of ERα, ERβ and AIB-1 and TIF-2 in MF of normal, benign proliferative disease (BPD) and carcinomas of breast.
Design: Tissue microarray (TMA) sections of 235 breast cancers, 62 BPD and 45 normal breast tissues were tested for the expression of ERβ1(AR385-5R, Biogenex; Ab288/14C8, ABCAM), AIB-1 (Clone 34, BD), TIF-2 (Clone 29, BD) and ERα (ID5, Dako) in MF. MF were identified by actin–SMA staining using EnVision G/2 Doublestain system (Dako, CA). Positive and negative controls and protein block serum–free were used. The percentage of nuclear/cytoplasmic expression was evaluated. Categorial variables were also analyzed.
Results: ERβ1, AIB-1 and TIF-2 were expressed in epithelial cells (EP) and stromal cells including MF and lymphocytes and mostly in nuclei. ERα was not expressed in MF. Expression of ERβ1 in MF was significantly associated with that of AIB-1 and TIF-2 with a statistical significance ($p<0.001$). ERβ1, AIB-1 and TIF-2 were more frequently expressed in cancer -associated MF and in high grade carcinoma with desmoplastic reaction and heavy lymphocytic infiltration. Nuclear expression of ERβ1, AIB-1 and TIF-2 in MF gradually increased from normal, through BPD, to carcinomas. The nuclear expression in EP in BPD and carcinoma was positively correlated with that in MF. Distinct cytoplasmic staining of ERβ1and TIF-2 in MF was noted more in carcinoma.

ERβ, AIB-1, and TIF-2 Expression in Myofibroblasts

	ERbeta	TIF-2(%)	AIB-1(%)
Normal EP	33 (15/45)	22.2 (10/45)	11.1 (5/45)
MP	27.7 (5/18)	16 (3/18)	5.0 (1/18)
BPD EP	45.6 (28/62)	45.6 (28/62)	25.8 (16/62)
MP	39.4 (15/38)	39.7 (15/38)	13.5 (5/38)
Carcinoma EP	70.2 (165/235)	61.7 (145/235)	38.3 (90/235)
MP	56.1 (55/98)	48.0 (48/98)	23.6 (23/98)

Conclusions: ERβ1, AIB-1 and TIF-2 expression in myofibroblasts correlated with degree of malignant transformation of breast tissue. Our findings suggests that ERβ1-dependent (genomic and non-genomic) and ER coregulator-dependent (AIB-1, TIF-2) signal transductions in myofibroblasts may be involved in the initiation and progression of breast carcinomas.

173 Triple-Negative Breast Cancers: Different Sensitivity to Chemotheray

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Background: Triple-negative breast cancers (TNBCs) and basal-like breast cancers (BLBCs) have been known as poor outcome subtypes and lack of targeted therapy. Previous studies have shown conflicting results regarding the difference of prognostic significance between TNBCs and BLBCs. In this study, we aimed to characterize the prognostic features of TNBCs, in view of BLBCs and quintuple-negative breast cancers (QNBCs).
Design: Using immunohistochemistry-based tissue microarray analysis, we categorized 951 primary breast cancers into four or five subtypes according to the expression of ER, PR, HER2, and basal markers (CK5/6, EGFR).
Results: The results of this study showed that both TNBCs and BLBCs were associated with high histological and/or nuclear grades. When the TNBCs are divided into two subtypes by the presence of basal markers, the clinicopathologic characteristics of TNBCs were mainly maintained in the BLBCs. After multivariate analysis, the QNBCs had a worse prognosis than the BLBCs, and worst prognosis among all five subtypes. Interestingly, the patients with BLBCs without chemotherapy treatment had a shorter disease free and overall survival than any other subtypes including QNBCs. With adjuvant chemotherapy, BLBCs were not the worst prognostic group anymore, indicating that BLBCs appear to respond better than QNBCs among TNBCs. BLBCs showed chemotherapy response in both anthracycline based drug regimen and CMF chemotherapy. On the other hand, QNBCs did respond to CMF but not to anthracycline based drug.
Conclusions: The BLBC subtype is a subgroup of TNBCs that has significant chemotherapy benefit, especially anthracyclines but the QNBCs are not. The QNBCs comprise 5.5% to 13.5% of all breast cancers of the published study subjects and these results call for caution in the identification of subgroup of patients for therapeutic classification.

174 Breast Cancer Molecular Subtypes Treated with Neoadjuvant Chemotherapy (NACT): Correlation with Grade, Ploidy, Ki67 and p53 Expression

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Background: The identification of tumor characteristics that can predict response to NACT is critical for determining outcome and response to chemotherapy in patients with breast cancer. The aim of this study is to determine if there is an association between molecular subtypes of breast cancer and pathologic response, grade, ploidy, ki67 and p53 expression.
Design: Patients with a diagnosis of invasive carcinoma on core biopsies who underwent NACT between the years 2000-2009 were identified in the electronic medical record and retrospectively analyzed. Clinical and pathologic data such as; race, age, clinical stage, tumor size, chemotherapy regimen, histologic type, tumor grade, and size of residual tumor was obtained. Tumor response was evaluated using Sataloff's classification: pathologic complete response (pCR); > 50%; < 50% reduction in tumor size and no therapy effect. Biomarkers (ER, PR, Her2Neu, ploidy, Ki67 and p53) were performed in a routine fashion and analyzed by the DAKO ACIS III image analyzer. Her2 positive cases were confirmed by FISH analysis. Tumors were stratified into four molecular subtypes; ER+PR±Her2-, ER+PR±Her2 +, ER-PR-Her2+, ER-PR-Her2-.
Results: There were 175 cases, of which 43 were excluded due to incomplete data. Age range was 21–77 yrs (mean 47). Of the 132 cases, 2 (1%) were stage I, 40 (31%) stage II, 47 (36%) stage III, 23 (17%) stage IV and 20 (15%) not known. Histologic types were ductal (95%) and lobular (5%) carcinoma. Chemotherapy regimens included adriamycin and cytoxan (AC) followed by taxanes in 62%, AC in 15%, Herceptin in 13%, 10% patients also received carboplatin, Gleevec and Gemzar. Complete response was noted in 18.9%.

Tumor profile	ER+PR±Her2- (50)	ER+PR±Her2+ (17)	ER-PR-Her2+ (26)	ER-PR-Her2- (39)
Grade 1 (1)	1	0	0	0
Grade 2 (61)	33	13	8	7
Grade 3 (70)	16	4	18	32
Aneuploid/multiploid (95)	28	14	25	28
Diploid (27)	19	3	1	4
Ki67*	38.39±3.69	31.82±4.89	55.19±5.4	66.0±3.59
P53*	12.6±3.98	22.1±8.63	40.32±8.62	55.71±7.35
pCR (25)	5	3	10	7
> 50% (46)	20	6	6	14
< 50% (31)	10	4	4	13
No response (31)	15	4	6	6

* mean±SEM; (total number)
Conclusions: Molecular subtypes of breast cancer can predict response to NACT. Of the tumors with pCR, 68% were of the Her2+ and triple negative subtypes. These tumors were likely to be high grade with high ki67 and p53 expression. Non-responsive tumors were likely to be hormone receptor positive, diploid with a lower Ki67 and p53 expression.

175 Endogenous Hormone Levels and Risk of Androgen Receptor Positive Breast Cancer: Results from the Nurses' Health Study

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Background: High circulating levels of androgens as well as high estrogen levels are associated with an increased risk of estrogen receptor (ER)+ breast cancer, particularly among postmenopausal women. While many breast cancers express androgen receptor (AR), the relationship between circulating hormone levels and the risk of development of an AR+ breast cancer has not been previously investigated. An understanding of this relationship could provide new insights into the risks for AR+ breast cancer.
Design: We constructed tissue microarrays (TMAs) from paraffin blocks of breast cancers from women enrolled in the Nurses' Health Study who had provided a blood sample for assessment of circulating hormone levels. TMA sections were immunostained for ER and AR. Any nuclear staining (>1%) for ER and AR was considered positive. Plasma samples were prospectively analyzed for endogenous levels of estradiol (E2) and testosterone (T) among postmenopausal women not currently taking postmenopausal hormones at the time of blood collection. We determined the relative risks of developing ER and AR positive breast cancers in relation to circulating hormone levels among 196 cases (compared with 787 controls).
Results: Overall, 82% of the breast cancers were AR+. The Table shows the relative risk for the development of breast cancer according to ER and AR status and the circulating E2 and T levels.

Relative risk of ER+ and AR+ breast cancer according to levels of circulating hormones

Circulating hormone level	ER+ breast cancer	AR+ breast cancer
	N=151	N=151
	OR (95%CI)	OR (95%CI)
Low E2/Low T	1.0 (REF)	1.0(REF)
Low E2/High T	2.7 (1.5-4.9)	1.8 (1.0-3.2)
High E2/Low T	2.0 (1.1-3.6)	1.3 (0.7-2.3)
High E2/High T	3.2 (1.9-5.4)	2.1 (1.3-3.4)

*Adjusted for matching factors (age, fasting status, time, month of blood draw)
Either high E2, high T or both were significantly associated with ER+ breast cancers, but significantly increased risk of AR+ cancers was only seen when circulating levels of T were high, (Low E2/High T p=0.04, High E2/High T p=0.0017).
Conclusions: We found that high circulating levels of testosterone were significantly associated with development of AR+ breast cancers, irrespective of estradiol levels. A better understanding of factors involved in the development of AR+ breast cancers may help in the identification of novel risk assessment and treatment strategies.

176 Outcome of Women with Ductal Carcinoma In Situ (DCIS) and Concurrent Lobular Neoplasia Treated with Breast Conserving Therapy: A Case-Control Study of 657 Patients from the Cancer Research Network

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Background: A variety of clinical and pathologic factors influence the local recurrence (LR) rate among women with DCIS treated with breast conserving therapy (BCT). However, the impact of concurrent lobular neoplasia (LN) on the risk of LR in women with DCIS treated with BCT has not been examined in detail.
Design: Among 2,995 women with DCIS treated with BCT between 1990-2001 at three HMOs in the Cancer Research Network, 657 were enrolled in a case-control study to assess clinical and pathologic factors associated with LR and had slides available for review. We examined the association between the presence of LN in these specimens and various clinical factors (age at diagnosis, presentation, family history), pathologic features of the DCIS (nuclear grade, architectural pattern, comedo necrosis, cancerization of lobules, stromal desmoplasia/inflammation), and the presence of concurrent atypical ductal hyperplasia and columnar cell lesions. We also examined the influence of LN

on the risk of LR in these patients. Relative risks were adjusted for matching factors, treatment, lesion size and margin status.
Results: Specimens from 169 of the 657 women with DCIS (26%) additionally demonstrated LN. The presence of LN was not associated with patient age, presentation, or family history of breast cancer. None of the pathologic features of DCIS were significantly associated with the presence of LN. However, LN was significantly associated with the concurrent presence of flat epithelial atypia ($p<0.001$) and atypical ductal hyperplasia ($p=0.001$). Overall, women with DCIS and concurrent LN had a 1.9-fold increase in their risk of local recurrence (95% CI 1.2-2.9) when compared with women without concurrent LN. When stratified by type of local recurrence, LN was significantly associated with DCIS recurrence (RR 2.3, 95% CI 1.2-4.2) but not with invasive recurrence (RR 1.3, 95% CI 0.7-2.6).
Conclusions: Among this population of women with DCIS treated with BCT, the presence of LN was significantly associated with the presence of other low grade precursor lesions. Moreover, the presence of concurrent LN conferred a greater than 2-fold increase in the risk of local recurrence of DCIS.

177 Columnar Cell Lesions and Subsequent Breast Cancer Risk: Results from the Nurses' Health Studies

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Background: Emerging evidence suggests that columnar cell lesions (CCL) represent an early stage in the low grade breast neoplasia pathway. However, the level of subsequent breast cancer risk conferred by these lesions when present in a benign breast biopsy is unclear.
Design: We examined the association between the presence of CCL and subsequent breast cancer risk in a case-control study of benign breast disease (BBD) and breast cancer nested within the Nurses' Health Studies (394 cases, 1606 controls). Benign breast biopsy slides were reviewed blinded to case-control status and categorized as showing non-proliferative lesions, proliferative lesions without atypia or atypical hyperplasia; the presence or absence of CCL was also noted. Logistic regression was used to compute odds ratios (ORs) and 95% confidence intervals (CIs) for the association between CCL and breast cancer risk. There were too few examples of CCL with atypia (i.e., flat epithelial atypia) to further stratify the analysis according to the presence or absence of atypia in the CCL.
Results: CCL were seen in 588 of the benign breast biopsies (140 cases, 448 controls). The presence of CCL was significantly associated with the presence of proliferative lesions without atypia and with atypical hyperplasia ($p<0.001$, for both). Women with CCL had an increased risk of breast cancer compared with those without CCL (OR=1.44, 95%CI 1.14–1.83). However, this increase in risk was attenuated after adjustment for histologic category of BBD (OR=1.20, 95%CI 0.94–1.54). The table shows the relative risk for development of breast cancer jointly classified by BBD category and the presence or absence of CCL.

BBD/CCL histologic category	Cases	Controls	OR (95% CI)
Nonproliferative, no CCL	80	518	1.0 (Reference)
Nonproliferative, CCL	19	93	1.33 (0.77-2.29)
Proliferative, no CCL	125	558	1.46 (1.08-1.99)
Proliferative, CCL	74	277	1.81 (1.27-2.58)
Atypical hyperplasia, no CCL	49	82	4.26 (2.75-6.59)
Atypical hyperplasia, CCL	47	78	4.37 (2.81-6.81)

BBD=Benign Breast Disease; CCL=Columnar cell lesions
Conclusions: In this case-control study, CCL were associated with a mild increase in breast cancer risk but the risk associated with these lesions did not appear to be independent of concurrent proliferative breast lesions.

178 P75/P63; a Novel Immunohistochemical Cocktail Which Is Diagnostically Superior to P63 for the Evaluation of Myoepithelial Cells in Challenging Breast Lesions

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Background: Nerve growth Factor receptor (NGFR/p75^{NTR}) a cell surface receptor glycoprotein with cytoplasmic/membrane staining has been identified as an adjunct marker of myoepithelial cells (MECS). p63 is an established myoepithelial cell nuclear marker. MECS may be attenuated/discontinuous in challenging breast cases. The aim of the study was to combine the cytoplasmic/membranous pattern of p75 with the nuclear pattern of p63 in a novel immunohistochemical cocktail developed to improve detection of MECS in breast lesions.
Design: p63/p75 cocktail was developed and optimized. 100 breast excision specimens were retrieved from the files with institutional approval. H&E slides were reviewed to confirm the diagnosis. Sections were stained for p63, p75 and the p75 cocktail and scored independently by 2 pathologists. A scoring system evaluating distribution and intensity in basal, luminal and stromal cells was designed as follows - Distribution: 1=<5% cells positive, 2= 5-25%, 3=25-50%, 4=>50%; Intensity: 0=negative, 1=weak, 2=moderate, 3=strong. P63 was compared to both p75 alone and the p75 cocktail.
Results: Malignant lesions (n=71) included: 30 invasive carcinomas (12 ductal, 7 lobular, 1 mucinous, 6 tubular, 4 microinvasive); 40 in situ lesions (35 DCIS (14 involving sclerosing adenosis), 5 LCIS); 1 encysted papillary carcinoma. Benign lesions (n=29) included: 12 complex sclerosing lesions, 5 epithelial hyperplasia, 5 papillomas, 4 columnar cell lesions, 2 syringomatous adenomas, 1 lactational adenoma. 93% DCIS had moderate/strong intensity MECS with p75cocktail vs 74% with p63. 94% DCIS had score 3 or 4 MECS distribution with p75cocktail vs 76% with p63. 100% LCIS had moderate/strong intensity MECS with p75cocktail vs 80% with p63. 80% LCIS had score 3 or 4 MECS distribution with p75cocktail vs 40% with p63. 29/30 invasive carcinomas showed complete absence of staining with p63 or p75 cocktail. 1/30 cases showed focal weak staining for all stains. In benign lesions intensity was equal for p63 and p75 cocktail, however the distribution was consistently greater with p75 cocktail (100% score 4) vs p63 (45% score 4). Focal weak stromal staining for p75 and p75cocktail was present in a minority of cases but did not limit evaluation.
Conclusions: p63/P75 cocktail is a novel, highly sensitive and reliable marker for evaluation of MECS in benign, premalignant and malignant breast lesions. It's characteristic continuous strong staining pattern offers diagnostic advantages over p63 alone.

179 Determining HER-2/Neu (H2N) Status on Needle Core Biopsies (NCBs) in the Neoadjuvant Setting: Does Florescence In-Situ Hybridization (FISH) Remain the Gold Standard for 2+ Cases in These Smaller Samples?

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Background: Emerging data show that patients with operable, H2N overexpressed/amplified breast carcinomas have significantly better responses (more frequently obtaining pathologic complete response and longer disease-free survival) when treated with trastuzumab simultaneously with neoadjuvant chemotherapy than with chemotherapy alone. With the increasing use of neoadjuvant therapies, clinicians require information on biomarkers including H2N status at the time of NCB. Currently cases staining 2+ by immunohistochemistry (IHC) are reflexed to FISH. Although many studies have shown excellent concordance between H2N status determined by IHC versus FISH in excised tumors, the question of whether FISH determined H2N status on NCBs in 2+ staining cases is equally reliable. The purpose of this study was to determine the accuracy of FISH determined H2N status on NCB material in 2+ cases by comparing with that assessed on subsequent EXBX from the same patient's tumor.
Design: 99 patients whose NCBs and subsequent EXBXs were pathologically evaluated at our institution were identified. For each patient, unstained sections from both specimens were prepared and used for IHC or FISH. IHC was performed using the HercepTest kit (Dako, Carpinteria, CA). Parallel unstained slides were used to perform FISH (dual probe, Vysis). Statistical analyses were performed on the resulting data.
Results: 14 of 99 (14%) cases demonstrated 2+ IHC staining in the NCB specimen. 8 of 14 (57%) were not amplified in both the NCB and EXBX whereas 3 of 14 (21%) were amplified in both specimens. The remaining 3 had discordant results (1 was amplified in the NCB but not amplified in the EXBX while 2 were not amplified in the NCB but amplified in the EXBX).
Conclusions: 78% of 2+ IHC staining NCB cases demonstrated concordant FISH results in the NCB and EXBX specimens. Performing FISH on NCB material in 2+ IHC cases yields a result that is tantamount to that done by FISH on the EXBX in the majority of cases.

180 Comparative Analysis of Breast Prognostic Indicators by Immunohistochemistry (IHC) and Oncotype DX (ODX)

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Background: ODX, a reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay of 21 selected genes performed in parafin-embedded tumor tissue, predicts the risk for distant recurrence among patients with lymph node negative, estrogen receptor (ER) positive breast cancer treated with tamoxifen. Patients with low recurrence score (<18) do not benefit from chemotherapy treatment. A comparative study of ER, PR and Her2 neu results obtained by IHC and RT-PCR (ODX) is performed.
Design: ER, PR, Her2-neu status defined by IHC and ODX were reviewed in 89 breast cancer cases collected between 2007 and 2009 from differents laboratories. Results of IHC and RT-PCR (ODX) were compared.
Results: Results from both assays are summarized in table1. Discrepancy was observed in 2 cases for ER(2%), 7 cases for PR (7%). No discrepancy was observed for Her2 when IHC and FISH results were combined. 2 ER negative cases by ODX showed weak (1-2+) positivity by IHC in less than 10% of tumor cells. 3 of 4 PR negative cases by ODX showed weak (1-2+) positivity by IHC in less than 50% of tumor cells. 3 of 11 PR negative cases by IHC were positive by ODX, however, the positivity was borderline (5.7-6.3). All 12 cases with equivocal Her2 positivity by IHC were negative by ODX and FISH. Also both cases with negative Her2 by IHC and equivocal positivity with ODX were negative by FISH.

Table 1

IHC	number of cases	ODX +	ODX -	ODX equivocal
ER +	88	86	2	
ER -	1	0	1	
PR +	78	74	4	
PR -	11	3	8	
HER2 +	5	2	2	1
HER2 -	72		57	2
HER2 equivocal	12		12	

Conclusions: IHC results are comparable to RT-PCR (ODX). Discrepancies were observed in only a minority of cases, occuring most often in PR results. Therefore, use of a costly test such as ODX is not necessary to determine the status of ER, PR and Her2-neu.

181 Stromal Caveolin-1 and 3 Expression as Predictive Markers in ER-Positive Primary Breast Carcinoma

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Background: Caveolin-1 and 3 are signature proteins of caveolae, specialized plasma membrane invaginations in mesenchymal cells. Caveolae are involved in

receptor-independent endocytosis, recruiting lipids and proteins for operation in signal transduction pathways. Recently, loss of stromal caveolin-1 expression in primary breast carcinomas has been associated with disease progression and metastasis, suggesting a role as a potential prognostic marker. In contrast, a lack of breast myoepithelial cell expression of caveolin-3, an isoform primarily found in skeletal and cardiac muscle, may confer a protective phenotype against the development of metastases and may result in a decreased tumor burden in translational studies. The aim of this study is to compare immunohistochemical (IHC) expression of caveolin-1 and 3 in ER-positive breast carcinomas with conventional predictive and prognostic markers.
Design: Primary ER-positive invasive breast carcinomas from resection specimens of 189 female patients diagnosed from 2006-2008 were included. Histologic and clinical data was collected for each case, including tumor size (cm), grade, type, number of axillary lymph node metastases, hormone receptor (ER, PR) and HER2 status, MIB-1 proliferation index, and Oncotype recurrence score. Tissue microarrays with two, 1-mm cores from each carcinoma were created and stained with monoclonal antibodies to caveolins-1 and 3, and resulting IHC expression was scored on a four-point scale.
Results: Epithelial caveolin-1 was negative for all but one of the carcinomas examined, with universal strong (3+) staining of endothelium and residual myoepithelial cells. Stromal caveolin-1 expression ranged from negative (14%) to 3+ (48%). Caveolin-3 IHC demonstrated no staining in tumor epithelium or stroma for all cases. Stromal caveolin-1 expression demonstrated no statistically significant association with tumor size (p=0.67), histologic grade (p=0.68), lymph node metastasis (p=0.25), MIB-1 proliferation index (p=0.25), or Oncotype score (p=0.46).
Conclusions: Caveolin-1 was not expressed in malignant epithelium but was present in peritumoral stroma in approximately 2/3 of the carcinomas. Caveolin-3 staining was negative in all cases. No significant association was found between stromal caveolin-1 expression and conventional predictive and prognostic markers. Continued investigation is warranted to assess caveolin expression in other subsets of breast carcinoma and to examine IHC correlation with long term clinical data including local recurrence, metastasis, and outcome.

182 Impact of Decalcification on Receptor Status in Breast Cancer

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Background: Metastasis of breast cancer to bone is observed in up to 70% of patients at post-mortem examination. Bone metastases in breast cancer, especially when solitary, may be amenable to surgical excision and radioablation in addition to systemic therapy. The pathology material obtained from bone specimens requires decalcification. The decalcification process may potentially affect the receptor status of the metastatic breast cancer and, therefore, impact the decision for systemic therapy. We conducted this pilot study to assess the reliability of receptor studies after decalcification.
Design: We prospectively selected 10 random cases of breast excision for cancer. In addition to the regularly proccessed tumor sections, one tumor section was subjected to 1 hour of decalcification following formalin fixation. Immunohistochemical studies for estrogen receptor (ER), progesterone receptor (PR) and her2 were performed on one representative section of the regularly processed tumor block and one decalcified tumor block. Three pathologists (FD, BS, JM) independently scored the slides. Scoring of ER and PR were performed according to the Allred scoring system (proportion 0-5 plus intensity 0-3 with a score range of 0, 2-8). Scoring of her2 was performed according to the Dako scoring system (0-3). The mean scores were used for comparison.
Results: The tumors were all invasive ductal carcinomas except case 3, which was an invasive lobular carcinoma (mean size = 1.8 cm). Six tumors were poorly differentiated and 4 were moderately differentiated. In all cases, the scores either dropped or remained unchanged after decalcification.

Receptor scoring

Case	ER score drop	PR score drop	her2 score drop	pT	pN	Receptor status
1	1	1	0	2	1	HR+/her2-
2	2	0	0	1c	1	HR+/her2-
3	2	1	1	1c	1	HR+/her2-
4	3	1	0	2	1	HR+/her2-
5	1	3	0	2	1	HR+/her2-
6	0	0	0	1b	0	her2+
7	0	0	1	1c	0	her2+
8	2	4	0	1c	1	TN
9	0	0	0	1c	0	TN
10	0	0	0	2	2	TN

ER: Estrogen receptor; PR: Progesterone receptor; HR: Hormone receptor; TN: Triple negative

The mean drop in the proportion of tumor cells staining was 21% for ER and 9.8% for PR. The mean drop in the intensity of staining in tumor cells was 1.4 for ER and 1.3 for PR. The mean drop in Allred scoring was 1.8 for ER and 2.0 for PR. The mean score drop for her2 was 1.0.
Conclusions: The immunohistochemical staining for ER, PR and her2 was more heterogeneous after decalcification with an overall reduction in the proportion and the intensity of staining.

183 Columnar Cell Lesions and Flat Epithelial Atypia in Breast Biopsies Performed Due to the Presence of Mammographic Suspicious Abnormalities

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Background: Columnar cell lesions (CCL) are characterized by the presence of columnar epithelial cells lining enlarged TDLUs of the breast. There is renewed interest in it because these lesions are been encountered with increasing frequency in excisional biopsies recommended by the mammographic finding of microcalcifications. The manner in which CCL are classified has varied among different authors. The aim of our study was to investigate frequency and types of CLL in breast biopsies performed due to the presence of mammographic suspicious abnormalities.
Design: We selected 249 breast biopsies required by suspicious mammographic findings, previously categorized as BI-RADS 3 or 4. Original H&E stained sections were reviewed and CCL were classified according to Schnitt S criteria as columnar cell change (CCC), columnar cell hyperplasia (CCH), or flat epithelial atypia (FEA).
Results: CCL were detected in 91/249 (36.5%) biopsies, being categorized as: CCC-81/91 (89.0%); CCH-4/91 (4.3%); FEA-6/91 (6.5%). CCL coexisted with epithelial hyperplasias in 70/91 (76.9%) cases (p value<0.05), 70% comprising usual ductal hyperplasia, 9% being atypical ductal hyperplasia, and 7% diagnosed as atypical lobular hyperplasia. We also detected benign neoplasias in 24/91 (26.3%) CCL cases and malignant neoplasias in 29/91 (31.8%) CCL cases (p value<0.05). Microcalcifications were identified in 124/249 (50.0%) specimens and were present within the CCL in 72/124 (58.0%) biopsies, being this association statistically significant (p value<0.05). Microcalcifications were exclusively present within malignant lesions in 30/124 (24.1%) biopsies. Besides CCL, we observed other benign lesions in 69/249 (27.7%) specimens, including ductal ectasia/cysts, apocrine metaplasia, sclerosing adenosis, and radial scar. Benign neoplasias were the sole diagnosis in only 2 biopsies, while malignant neoplasias were noted in 95 cases.
Conclusions: Our results showed that the majority of CCL are represented by lesions without cytologic atypia or architectural complexity which frequently coexisted with other lesions. The coexistence of CCL and invasive carcinomas was statistically significant (p value<0.05). We also found that CCL were the main site of microcalcifications either mammographically or histologically detected (p value<0.05). Most importantly, microcalcifications associated with these lesions allowed the incidental diagnosis of clinically silent carcinomas present elsewhere within the specimens.

184 Automated FISH Analysis for the HER2 Gene Status Assessment in Breast Cancer: A Validation Study Based on 306 Cases

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Background: Automatic analysis of HER2 fluorescence-in-situ-hybridization (FISH) samples have been recently developed as a potential alternative to conventional gene assessment based on manual signal enumeration. However, specific studies addressing the evaluation of automated systems and their clinical validation are limited. We present a comparative study of traditional/manual versus software-assisted HER2 FISH scoring applied to routine breast cancer samples.
Design: HER2 FISH was investigated by analysis of 356 breast tumor samples obtained from routinely handled tissues, including core needle biopsies (n=188; 52.8%) and surgical specimens (n=168, 47.2%). FISH was performed using commercial probes (PathVysion®) and analyzed according to ASCO/CAP guidelines. The commercial software Metafer4 (Metasystems, Altlussheim, Germany) was used for automated analysis. For conventional evaluation, a tumor was considered to be amplified when the ratio of HER2 gene signals to chromosome 17 was greater than 2.2, non-amplified when the ratio was less than 1.8, and equivocal when the ratio was in the range 1.8-2.2. The Metafer4 PathVysion V2 classifier defines the range as 1.5-3.0 for borderline cases, and the ratio as less than 1.5 and greater than 3.0 for non-amplified and amplified cases, respectively.
Results: An average of 42 cells and 385 tiles for each sample was analyzed with manual and automated counting, respectively. Fifty tissue samples (50/356, 14.0%) with excess tissue autofluorescence and weak hybridization signals were excluded from the analysis. Manual and automated analysis was concordant for 286/306 cases (93.4%): discrepancies were retrospectively explained with suboptimal tumor area selection, HER2 tumoral heterogeneity or neoplasms with a ratio close to the equivocal range. The concordance rate was 97.7% and 91.8%, respectively, for non-amplified and amplified cases, whereas for equivocal cases, it was 57.1%. Borderline cases were 14/306 (4.5%) for manual counting and 21/306 (6.9%) for automated scoring. The concordance rate was near total (99%) for manual and automated scoring if equivocal cases were excluded.
Conclusions: Automated analysis of FISH samples for HER2 testing allows a faster and accurate study of a larger number of cells compared with that obtained by visual counting, especially for non-amplified and amplified cases. Although manual counting is still needed for equivocal cases, our data indicate that an automated approach to HER2 FISH scoring may be implemented in routine practice.

185 High Expression of EIG121 Identifies Women with Triple Negative Breast Cancer with a Better Prognosis

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Background: Triple negative breast cancer is the most clinically aggressive form of breast cancer. Because they are defined by lack of ER, PR, and HER-2/neu, it is acknowledged that triple negative tumors are heterogeneous. We discovered the novel gene EIG121 from a microarray of baseline and post-treatment endometrial biopsies from women taking estrogen-based hormone replacement therapy. EIG121 is a lysosomal protein up-regulated in endometrioid-type endometrial carcinoma, the histotype most closely associated with unoppposed estrogen exposure. In fact, EIG121 is the single best gene to discriminate endometrioid carcinoma (good prognosis) from non-estrogen dependent non-endometrioid endometrial carcinoma (bad prognosis). We hypothesized that EIG121 may stratify patients with triple negative breast cancer into distinct prognosis groups.
Design: EIG121 expression was quantified using reverse phase protein lysate array (RPPA) in 460 breast cancers, 127 of these triple negative cases. RPPA is a high-throughput "dot blot" in which dilutions of protein lysate are spotted on nitrocellulose slides. Each slide is probed with a different monospecific antibody. A DAKO-catalyzed signal amplification system is used for signal detection. An advantage over immunohistochemistry is that RPPA is quantitative rather than qualitative. A tetracycline-inducible in vitro cell system was generated to analyze the effect of

EIG121 in EGFR-mediated cell signaling, as EGFR is expressed in the majority of triple negative breast cancers.
Results: Overall, expression of EIG121 was low in triple negative tumors compared to ER+ cases. However, EIG121 expression was sufficiently heterogeneous to allow for stratification into EIG121 high and EIG121 low groups. The EIG121 high group had significantly increased recurrence free survival (hazard ratio 0.477; p=0.002) and overall survival (hazard ratio 0.565; p=0.032) compared to the EIG121 low group. EIG121 was significantly and negatively correlated with levels of EGFR, pEGFR, AKT, and pAKT. In the cell-based system system, EIG121 was shown to bind to and degrade EGFR.
Conclusions: High expression of EIG121 identifies triple negative breast cancer patients with improved survival. Breast cancers that over-express growth factor receptors are known to be biologically more aggressive. Therefore, the pro-survival effect of high EIG121 may be due, in part, to EIG121 directly inhibiting EGFR expression by lysosomal-mediated degradation.

186 Effect of Formalin Fixation Time on Her 2 Expression by Immunohistochemical Technique

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Background: The 2007 American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for Her 2 Testing in Breast Cancer include the specification that formalin fixation for fewer than 6 hours or longer than 48 hours is not recommended. Although there is evidence to suggest that pre-fixation tissue handling, type of fixative and fixation time may have an effect on Her2 expression levels as detected by immunohistochemistry, the reason for the choice of 6 and 48 hours for the minimum and maximum formalin fixation times is unclear.
Design: Using the same Her2 over-expressing cell line used to validate the Herceptest, SK-OV-3, we attempt a systematic study of the effect of formalin fixation time on detection of Her2 overexpression by immunohistochemical (IHC) technique. SK-OV-3 cells grown in tissue culture were pelleted and fixed in formalin for times ranging from 10 days to 25 minutes. The formalin fixed cell pellets were then processed according to the our department's standard breast biopsy protocol. Slides were scored for intensity and pattern of Her2 expression.
Results: The overexpression of Her2 in the SK-OV-3 cell line was confirmed using both FISH and immunohistochemical techniques. FISH testing demonstrated the ratio of Her2 to chromosome 17 to be 2.8. SK-OV-3 cell pellets incubated for long (1, 2, 3, 10 day) formalin fixation times showed no significant difference in intensity or proportion of cells exhibiting strong membrane staining by IHC. Cell pellets incubated for 6 hours or less showed a decrease in stain intensity and proportion of cells with membrane staining.
Conclusions: Her2 cell surface receptor as detected by IHC appears to be relatively unaffected by length of formalin fixation time greater than 24 hours. Formalin fixation less than 6 hours may decrease the sensitivity of Her2 testing by IHC. Although we recognize that tissue culture cell pellets do not exactly recapitulate the cellular environment of invasive carcinomas, the use of a cell line standard can help to identify whether weakness and inconsistency in Her2 scoring systems lie in the heterogeneity of formalin fixation times or are instead a reflection of heterogeneity of tumor composition or other aspects of tumor tissue handling.

187 Breast Cancer Tumor Marker Profile: A Comparison of Clinical Laboratory Testing Methods

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Background: Both morphology and molecular based techniques have been used to measure tumor markers in invasive breast cancer. Immuno-histochemistry (IHC) is currently the standard method to measure estrogen (ER) and progesterone (PR) receptors. There has been excellent correlation between gene copy number of HER-2/neu determined by fluorescence in situ hybridization (FISH) and protein expression by IHC. The high sensitivity and specificity of molecular biology based tests (RT-PCR) offer great potential to individualize treatment and further fulfill the promise of personalized medicine. In this analysis, we compare the sensitivity and specificity of different testing methods for ER, PR and HER-2/neu and we correlate the findings of IHC and FISH tests with RT-PCR results.
Design: We collected 39 invasive breast cancer samples for which tumor marker status (ER, PR and HER-2/neu) was available by 2 or 3 different testing methods: IHC and RT-PCR for ER and PR and IHC, FISH and RT-PCR for HER-2/neu. We calculated sensitivity and specificity for IHC and FISH tests correlating RT-PCR as the gold standard. RT-PCR data was collected from OncotypeDx report (Genomic Health Inc). Student's t test was used to correlate the results by different testing methods.
Results: In our laboratory, both the sensitivity and specificity of test results by IHC and FISH for ER and HER-2/neu were 100% taking RT-PCR as the gold standard .There was also 100% correlation for ER and HER-2/neu when compared with RT-PCR results . There were three false positive cases for PR expression again taking RT-PCR as the gold standard with a sensitivity of 92% and a specificity of 100%. It is important to note that all three false positive PR tumor samples showed low level PR expression (Allred score of 4, 4 and 6). There is a statistically significant relation between low level PR expression by IHC and negative RT-PCR results for PR ($p<0.001$) in this analysis.
Conclusions: There is complete correlation between ER and HER-2/neu expression by different testing methods (IHC and RT-PCR for ER and IHC, FISH and RT-PCR for HER-2/neu) with 100% sensitivity and specificity. There were 3 false positive PR expression by IHC with a sensitivity of 92% and a specificity of 100%. We found that low level weak PR expression by IHC is correlated with negative PR results by RT-PCR ($p<0.001$). In conclusion, in selected cases RT-PCR can be used to confirm the tumor marker expression to plan targeted therapy.

188 ER/PR and Her2/Neu Correlation between Immunohistochemisty and Gene Expression Profiling in Women with Invasive Breast Cancer

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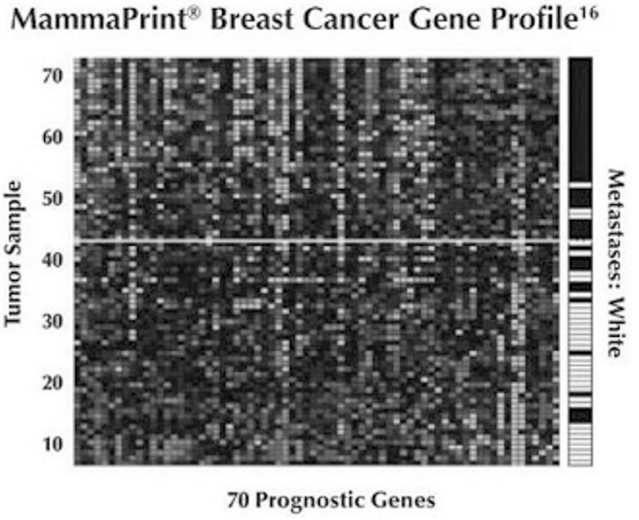
Background: Mammaprint® is a 70 gene FDA approved gene expression profile used to stratify invasive breast cancer patients into recurrence risk categories using fresh tissue(Figure 1). This test also produces ER/PR and Her-2/Neu gene signatures. The purpose of this pilot study was to correlate the results of routine quantitative immunohistochemisty (IHC) of ER/PR and Her2/Neu protein expression to that of Mammaprint® gene expression profiling.



Design: Fresh tissue was sent for Mammaprint® analysis on 29 patients treated at the University of South Florida Breast Health Center with suspected or biopsy proven diagnosis of invasive breast cancer from 6/09 through 9/09. Fresh tissue samples were obtained by core biopsy or at the time of surgical excision. A gene expression profile for ER/PR and Her-2/Neu was included in the analysis where tumor volume was sufficient (≥ 30% tumor/connective tissue ratio). The remainder of the tissue was routinely processed for pathologic analysis and evaluation of ER/PR and Her-2/Neu status was performed by IHC. ER/PR was considered positive for ≥ 1% positive nuclear staining.
Results: ER/PR and Her2/Neu data were obtained for both Mammaprint® and IHC for 15 patients. Thirteen out of 15 (87%) cases were ER/PR concordant when comparing IHC to Mammaprint® results (Table 1). The two discordant cases were ER/PR positive by Mammaprint® and ER/PR negative by IHC. All cases were Her2/Neu concordant (Table 2).

IHC vs Mammaprint

		Mammaprint	
		ER/PR +	ER/PR -
IHC	ER/PR +	15	0
	ER/PR -	2	0

Table 1

IHC vs Mammaprint

		Mammaprint	
		Her-2/Neu +	Her-2/Neu -
IHC	Her-2/Neu +	2	N/A
	Her-2/Neu -	N/A	15

Table 2

Conclusions: This study indicates a high but not uniform concordance between IHC and fresh tissue gene expression profiling and suggests that gene expression profiling performed on fresh tissue may be more sensitive for the detection of ER/PR status. When discordant results between these two methodologies arise, it is unclear how clinicians may interpret the data and what effect interpretation may have on therapeutic decisions, prognosis and outcome. Larger studies with long-term follow up may answer these clinically relevant questions.

189 Extravasated Mucin Pools in Breast Core Needle Biopsy: Ten Year Experience at a Single Institution

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Background: Extravasated mucin (EM) in core needle biopsy (CNB) is a worrisome finding and routinely leads to surgical excision (EXC). Because EM alone is uncommon in CNB, data on the excisional findings in cases with only benign or atypical epithelium is limited.
Design: We reviewed CNBs with EM diagnosed between 2000 and 2009 together with the subsequent EXC, with the exclusion of malignant cases. We also collected patient demographic, radiological and follow-up (FU) information. Immunoperoxidase stain for MUC6 was performed on 20 CNBs and 22 EXCs.
Results: We identified 30 patients (pts) with EM on CNB. All pts were women, with median age of 50 yrs (range: 39-78). Calcifications (Ca++) had prompted CNB in 28 pts (93%), and were found in EM in 9 cases, within acini in 23 and in the stroma in 4. The CNB had been done for a mass lesion in 2 pts. The epithelium adjacent to EM was benign (EM-BEN) in 12 cases and atypical (EM-ATP) in 18 (9 ADH, 5 columnar cells with atypia, 2 ALH, 1 atypical apocrine cells, 1 suspicious for carcinoma). Deeper levels had been originally obtained in 8 cases (27%), of which 6 had atypia. EXC of 22 cases (73%) was performed at our center. Only 1 EM-BEN CNB showed rare atypical

apocrine cells (AAC) in the EXC, in contrast to 5 EM-ATP that were upgraded to DCIS (4) and mucinous carcinoma (1.3 cm, intermediate nuclear grade). DCIS was solid-cribriform type with mucin (1), cribriform (1), solid (1), and micropapillary (1); nuclear grade was low (3) or intermediate to high (1). EM was present in the biopsy site in 8/22 EXC (36.6%), including 7 with atypical/malignant findings. Two pts with EM-ATP underwent EXC elsewhere and slides were not available for review. The radiological FU of a third patient remained unchanged at 6 months. Three EM-BEN pts were lost to FU, and 2 had benign mammograms at 32 and 33 months. Results of MUC6 stain were noncontributory due to loss of the EM on the deeper sections.

Results of Follow-up Excisions

	CNB	EXC	UPGRADE
EM-BEN	12	7/12 (58.3%)	1/7 (14.2%)
EM-ATP	18	15/18 (83.3%)	5/15 (33.3%)
TOTAL	30	22/30 (73.3%)	6/22 (27.2%)

Conclusions: The results of our study show that when EM alone is identified in a CNB and the histologic findings correlate with radiology, the EXC is usually benign. In these cases, CNB sampling of the area may suffice. In contrast, EM-ATP in CNB correlates with a more serious lesion in a third of cases and should always warrant excision.

190 Needle Core Biopsy Has High Sensitivity for Identifying Breast Papillary Lesions Requiring Surgical Excision: Diagnostic Accuracy in 161 Cases

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Background: Accuracy of needle core biopsy (CBx) to identify breast papillary lesions (PL) that require surgical excision is controversial. Our objective was to assess accuracy of CBx using 3 thresholds that showed substantial interobserver agreement (kappa 0.72-0.77).

Design: CBx cases with PL were identified from the pathology database. CBx with ADH, DCIS or invasive carcinoma (IC) adjacent to the PL were excluded. At least 2 pathologists independently reviewed each CBx and excision using a diagnostic algorithm and resolved discrepancies by consensus. Pathologists were masked to the excision when reviewing the CBx, and vice versa. Primary outcome was surgical excision with at least ADH (ADH+): papillomas with ADH or DCIS, intraductal papillary carcinoma (IDPC), or IC.

Results: Pathology review identified 164 CBx with PL. There were 151 (92%) excisions, 10 (6%) had clinical follow-up only, and 3 (2%) patients were lost to follow-up. Excisions showed 91 benign papillomas with or without UDH, 7 atypical papillomas, 11 papillomas with DCIS, 17 IDPC, 20 IC, 5 benign breast tissue with no residual lesion.

CBx Threshold	No.	Test performance for identifying PL with ADH+ on excision					
		False +	False -	Sensitivity	Specificity	PPV	NPV
Papilloma >10% UDH	89	36	3*	0.95	0.62	0.60	0.95
Papilloma ≥50% UDH	73	21	4**	0.93	0.78	0.71	0.95
Papillary lesion ADH+	62	12	6***	0.89	0.87	0.81	0.93

*1 atypical papilloma, 1 IC & 1 DCIS adjacent to papillomas with UDH; **same, plus 1 papilloma with DCIS, ***plus 1 IC adjacent to papillomas with UDH, 1 papilloma with DCIS

Test indices were similar when patients with clinical follow up were included, and also when the excision showed DCIS or worse, but excluded ADH. Sensitivities are higher if only histologic interpretation of the PL is considered, and cases where radiologic sampling missed adjacent IC and DCIS are not attributed to pathology error.

Conclusions: Excising all PL diagnosed by CBx ensures that all IC and DCIS are excised (sensitivity 100%). However, if 95% sensitivity is acceptable, substantial unnecessary surgeries may be averted if papillomas with no or minimal UDH on CBx do not undergo immediate excision. Follow-up mammography for these cases may safely identify the few additional women requiring surgery.

191 Diagnostic Utility of WT-1, Ki 67 and CD117 in Fibroepithelial Lesions of the Breast

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Background: Definite diagnosis of fibroepithelial lesions (FEL) as fibroadenoma (FA) or benign phylloides tumor (BPT) in core needle biopsies (CB) is difficult in some cases. Although both tumors are benign discrimination between both tumors is crucial as FA is usually managed by follow up or simple enucleation in contrast to BPT which is managed by adequate excision. The latter requisites free margin of at least 1 cm. To the best of our knowledge no definite clinical or imaging parameter can discriminate accurately between the two tumors. Presently, molecular and immunohistochemical techniques play a limited role in the diagnosis of FEL.

Design: Forty five FEL diagnosed in CB were prospectively studied. None of these cases showed necrosis, prominent cellular pleomorphism, stromal overgrowth, brisk stromal mitotic activity, leaf like pattern, entrapped native epithelial or fibroadipose tissue or heterologous elements. Immunohistochemistry using WT-1, Ki 67 and CD117 were applied on CB. In each case pattern and intensity of staining and expressing cells were assessed. Finally subsequent excision was reviewed.

Results: All the forty five patients were female, with a mean age of 41 years. Each patient had a palpable mass or mammographic finding indistinguishable from FA. On imaging tumor size ranged from 5 mm to 28 cm. All cases were diagnosed on CB as benign FEL, with a comment favoured FA in forty cases. Subsequent excision revealed FA in twenty nine cases: ten were cellular; two were complex and seventeen were FA, NOS. The remaining sixteen cases showed phylloides tumor (PT), three of which were borderline and thirteen were benign. WT-1 immunohistochemical stain showed stromal cytoplasmic expression in the proliferating spindle cells in all cases of PT. Negative expression was noted in the native breast tissue. All the cases of FA were negative identical to the native breast tissue. Low Ki 67 proliferation index ranged from 0%-15% was noted in both FA and PT. No statistical significance was present between the two neoplasms. All the cases included in the current study were negative for CD117.

Conclusions: The current study shows that immunohistochemistry using WT-1 antibody applied on CB is useful tool that aids in further classification of difficult cases of FEL, and thus guide management of these cases, in contrast to CD117 and Ki 67 antibodies.

192 Validation of the Magee Study Equation in Prediction of Breast Cancer Recurrence Risk Category by Oncotype DX™

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Background: Oncotype DX™ is a commercially available RT-PCR-based assay that provides a Recurrence Score (RS) and has been shown to provide prognostic and predictive information in ER-positive lymph node-negative breast cancer. The RS is divided into 3 risk categories as low, intermediate and high risk of tumor recurrence at 10 years. It has been recently shown that the RS can also be estimated by traditional histopathologic variables in approximately 2/3rd of cases using the Magee study equation (MSE) RS =13.424 + 5.420 (nuclear grade) + 5.538 (mitotic count) - 0.045 (ER immunohistochemical score) - 0.030 (PR immunohistochemical score) + 9.486 (HER2/neu) (Mod Pathol 2008;21:1255-61).

Design: The histopathologic variables of 154 cases were scored by 2 pathologists (GA and NNE) blinded to Oncotype DX™ results. The MSE was used to determine the risk category in all cases. The risk category obtained by the equation and the Oncotype DX™ RS were then compared.

Results: The concordance between the risk categories based on Oncotype DX™ RS and the RS using the MSE was 72% (111/154). Of the 43 discordant cases, 42 showed 1-category discordance and one case had 2-category discordance. Among the discordant cases, the mean and median differences in RS by the MSE vs Oncotype DX™ were 4.13 and 6.15, respectively (range 0.7-18.1). Of the discordant cases, 55.8% of the cases had a <10 RS point difference. The risk category in 17/43 discordant cases (39.5%) was altered by a RS margin of <2.

Comparison of risk categories using the Magee Study Equation vs. Oncotype Dx

	High risk using MSE†	Intermediate risk using MSE†	Low risk using MSE†	Total
High risk on ODX*	9	5	0	14
Intermediate risk on ODX*	2	18	25	45
Low risk on ODX*	1	10	84	95
Total	12	33	109	154

*ODX=Oncotype DX™, †=Magee study equation

Conclusions: This is the first study to independently validate the Magee study equation. This validation study confirms that the Oncotype DX™ RS is heavily influenced by the level of tumor hormone receptor expression, proliferation rate, nuclear pleomorphism, and HER2 status. Traditional histopathologic variables can thus be judiciously utilized in treatment decisions and Oncotype DX™ testing should be reserved for more complicated cases only.

193 Establishment of the Australian In Situ Hybridisation Program for the Assessment of HER2 Amplification in Breast Cancer: A Model for the Introduction of New Biomarkers into Clinical Practice

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Background: In August 2006 the Australian government announced a decision to subsidise trastuzumab therapy for early breast cancer, to commence six weeks later. It was mandated that HER2 gene amplification, determined by in situ hybridisation (ISH), be demonstrated, and that the sponsor company, Roche Products Pty Ltd, should fund this testing. This announcement potentially required provision of ISH testing for HER2 for every newly diagnosed breast cancer, where previously HER2 testing had been based on immunohistochemistry with support from a single fluorescent ISH (FISH) reference laboratory for indeterminate cases.

Design: The Australian HER2 Testing Advisory Board, an independent expert group, responded to the challenge of rapidly providing accurate nationwide ISH testing. Bright field ISH was selected as the testing platform and a decentralised testing model, with support from a central FISH laboratory, was adopted. An implementation plan was developed addressing standards for training, accreditation and quality assurance.

Results: Within six weeks eight pathology laboratories were accredited for ISH testing and by September 2008, two years after the announcement, 22 ISH testing laboratories were taking part in the national program and almost 20,000 ISH tests had been performed.

Conclusions: This abstract describes the design and rapid implementation of a nationwide program of bright field ISH as the first-line testing platform for HER2 status in early breast cancer. We believe this model for the coordinated and large scale implementation of a new biomarker test has wider application, given that accurate assessment of a range of novel biomarkers is being used increasingly to determine eligibility for new targeted treatment modalities.

194 Lobular Neoplasia on Breast Core Biopsy: Is Routine Excision Indicated? A Study of 353 Cases

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Background: Lobular neoplasia (LN) is regarded as a risk indicator for the development of invasive ductal or lobular carcinoma of the breast.The significance of these lesions on core biopsy is controversial, with relatively small sample sizes existing in the literature,and thus the utility of subsequent excisional biopsy (SEB) is debatable.

Design: A computer-based retrospective search was performed for a period of 43 months (January 2006 - July 2009) to retrieve cases of atypical lobular hyperplasia (ALH) and lobular carcinoma in-situ (LCIS) on core biopsy specimens. Exclusion criteria included a synchronous diagnosis of invasive carcinoma (IC) and/or ductal carcinoma in-situ (DCIS).Radiologic findings and data from SEB reports were recorded.Statistical analysis was performed by the Chi-square test using SAS 9.1 software.

Results: 278 cases of ALH, including 73 with atypical ductal hyperplasia (ADH), and 129 cases of LCIS, including 34 cases with ADH on core biopsy were identified in our database.Cases without subsequent surgical follow up were excluded, yielding 240 cases in ALH group(mean age 55.8 years) and 113 cases in LCIS group(mean age 53 years) for analysis.The mean period between core biopsy and SEB was 1.5months .Radiological findings included calcifications (262 cases), mass (65 cases),others (28 cases).DCIS and/or IC were detected at excision in 22.2% (20/90) and 10.3% (27/263) in LN/ADH and pure LN groups, respectively(P=0.004).

Table 1 . Subsequent Excisional Biopsy Findings

	Core Biopsy Diagnosis				
Highest-grade diagnosis on excision	ALH N= 178 (%)	ALH/ADH N=62 (%)	LCIS* N=85 (%)	LCIS/ ADH N=28 (%)	Total N=353
LCIS	36(20.2)	5(8.1)	44(51.8)	5(17.9)	90(25.5)
LCIS/ADH	6(3.4)	10(16.1)	12(14.1)	4(14.3)	32(9.1)
ADH	26(14.6)	26(41.9)	4(4.7)	5(17.9)	61(17.3)
DCIS	8(4.5)	5(8.1)	4(4.7)	5(17.9)	22(6.2)
Invasive ductal CA	3(1.7)	2(3.2)	5(5.9)	3(10.7)	13(3.7)
Invasive lobular CA	1(0.6)	3(4.8)	5(5.9)	2(7.1)	11(3.1)
Mixed ILC+IDC	1(0.6)	-	-	-	1(0.3)
Benign or ALH	78(43.8)	11(17.7)	11(12.9)	4(14.3)	104(29.5)

* 2/8 cases of pleomorphic LCIS showed ILC on SEB.

Conclusions: To our knowledge, this is the largest retrospective study on the risk of subsequent neoplasia on excision after a diagnosis of LN on core biopsy. While the risk of DCIS and/or IC on excision is significantly higher in LN with ADH compared with LN alone, the latter is still associated with a significant risk of DCIS and/or IC on excision. These results strongly support that excision is indicated after a diagnosis of LN on core biopsy.

195 HER2 Diagnosis Program in Argentina with a Standardized and Reproducible Technique: Variation in Positive Rates throughout the Years

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Background: HER2 testing has a key role in the management of breast cancer, so we settled a National testing Program to permit access to standardized Her 2 detection all over the country. In August 2003, 13 pathologists formed a cooperative group and created a national framework to train and set HER2 diagnostic centres in each country region. A coordinator responsible for coaching and evaluating centres and two technical consultants in charge of quality control and technique standardization were designated. External controls (i.e. CAP, Nordi QC, UK-NEQAST) were also implemented. In February 2004, the program was launched **Aim:** Report our findings from February 2004 to July 2009.

Design: HER2 over-expression was analyzed by Inmuno-Histo-Chemical (IHC) using policlonal antibody anti Her 2 (DAKO), microwave antigenic recovery, detection system EnVision (Dako) and developed with diaminobenzidine. Results were interpreted as hercepTest® guideline's Following ASCO-CAP recommendations HER 2 equivocal, were re-tested with FISH.

Results: 27097 IHQ tests were performed and 773 were re-tested by FISH: 343 (44%) amplified.

HER 2 over-expression

Year	0 n (%)	1 n (%)	2 n (%)	3 n (%)	Total
2004	590 (43.8)	426 (31.62)	50 (3.7)	281 (20.86)	1347
2005	1027 (48)	602 (28)	156 (7)	349 (17)	2134
2006	2407 (53.5)	1103 (24.5)	261 (5.8)	729 (16.2)	4500
2007	3327 (51.9)	1711 (26.7)	313 (4.9)	1064 (16.6)	6415
2008	4234 (53.39)	2060 (25.98)	376 (4.74)	1260 (15.89)	7930
2009	2695 (54.49)	1189 (24.92)	230 (4.82)	657 (13.77)	4771

Conclusions: HER2 National Program enabled country-wide HER2 testing. HER2 prevalence in our population drops off along the years and might be linked to the increase of early breast cancer testing and technique standardization. Internal quality control, done by the Advisory Board, as well as independent and external quality control, carried out by the CAP, NordiQC and UK-NEQAST have assured accurate outcome We presented performance, interpretation, and technical issues guides for HER2 testing based on our experience, endorsed by Argentinean Society of Pathology.

196 Estrogen Receptor β Isoforms 1, 2 and 5 and Oncotype Recurrence Score

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Background: The use of selective estrogen receptor modulators (SERMs) is a mainstay of breast carcinoma treatment. The ER receptor is a member of the steroid/thyroid nuclear receptor superfamily and is composed of an α and β subtype. The β subtype is further subdivided into five isoforms, ERβ1 through ERβ5. Isoforms ERβ1, ERβ2, ERβ4 and ERβ5 are breast specific while ERβ3 is testes specific. When evaluated in breast carcinoma, ERβ1 showed no association with disease outcome, while ERβ2 and ERβ5 correlated with improved relapse-free survival. ERβ2 is felt to be a prognostic indicator in breast carcinoma and is postulated to play a role in carcinogenesis. Oncotype DX is a reverse transcriptase polymerase chain reaction (RT-PCR) assay that predicts ten year risk of recurrence in lymph node negative ER-positive breast carcinomas. It utilizes the expression of twenty-one genes to give a Recurrence Score from 0-100. Patients are stratified into three risk categories; low (0-17), intermediate (18-30) and high risk (31+) of recurrence based on the Recurrence Score.

Design: One hundred thirty patients with known positivity for estrogen receptor alpha (ERα) and Recurrence Score available, were selected. Immunohistochemistry for ERβ1, ERβ2 and ERβ5 were each performed on three tissue microarrays with two 1mm cores of each tumor. Less than ten percent expression was used as the negative cutoff for each value. Results were compared to Recurrence Scores.

Results: Three percent of the carcinomas were ERβ1 positive, forty-eight percent were ERβ2 positive and sixty-six percent were ERβ5 positive.

ER β isoform and Recurrence Score

	Oncotype Low	Oncotype Intermediate	Oncotype High	p-value
ER β 1	2/130 = 1.5%	2/130 = 1.5%	0/130 = 0%	0.1667
ER β 2	39/130 = 30%	21/130 = 16%	3/130 = 2.3%	0.0000
ER β 5	46/130 = 35.4%	33/130 = 25.4%	7/130 = 5.4%	0.0000

Conclusions: In the ERα positive population studied, there is a statistically significant difference between Recurrence Score for ER β isoforms 2 and 5. ER β1 had too few positive cases to detect a difference between Recurrence Score risk groups. Positivity for ER β2 and ER β5 is thus associated with low and intermediate Recurrence Scores, and hence, with improved relapse-free survival.

197 Invasive Apocrine Carcinomas Are Molecularly Diverse Group of Breast Cancers

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Background: Extensive apocrine differentiation (large epithelial cells with prominent eosinophilic, flocculent cytoplasm, sharply defined cell borders, and with large nuclei containing prominent macronucleoli) is seen in approximately 5% of all breast cancers. Recent gene expression studies recognized a characteristic "molecular apocrine" gene expression profile in these tumors, different from common luminal and basal-like breast carcinoma subtypes. However, a practical, routine diagnostic and prognostic sub-classification of apocrine carcinomas is not known.

Design: A cohort of 55 female patients with invasive apocrine carcinomas was evaluated for the expression of estrogen receptor-alpha (ER), progesterone receptor (PR), androgen receptor (AR), Her-2/neu, and EGFR using routine immunohistochemical methods. Additionally, *HER2* and *EGFR* gene copy number was assessed using fluorescent in-situ hybridization (FISH).

Results: Of 55 cases, thirty-eight (69.1%) expressed a characteristic steroid receptor profile (ER-, PR-, AR+) of pure apocrine carcinoma (PAC). The remaining 17 cases (30.9%) lacked this specific profile (apocrine-like carcinomas, ALC): ALC with ER+/AR- phenotype (3 cases), ALC with ER-/AR- phenotype (4 cases), and ALC with ER+/AR+ phenotype (10 cases). Her-2/neu protein overexpression (3+) was observed in about half of the cases in the entire cohort (53.7%) without significant difference between the PAC and ALC groups (56.8% vs. 47.1%, p=0.81). EGFR was expressed (1+ to 3+) in 61.9% of all cases. A significantly higher proportion of PACs was positive for EGFR protein in comparison with the ALC subgroups (76.3% vs. 29.4%, p=0.006). A statistically significant inverse correlation between EGFR and Her-2/neu expression was seen in the PAC (p=0.006, r=-.499). *HER-2/neu* FISH results were concordant with Her-2/neu immunohistochemistry results in 92.5% cases. *EGFR gene* amplification was a rare event, present only in three (2 PAC and 1 ALC tumors) of 44 cancers (6.8%)

Conclusions: Breast carcinomas with apocrine differentiation are very heterogeneous in molecular terms. 52.6% of PAC cases can be classified as HER-2-overexpressing, and 47.4% as triple-negative breast carcinomas. 94.4% of triple negative PAC overexpressed EGFR and could accordingly be classified as basal-like breast carcinoma. A majority (76.5%) of ALC belonged to the luminal group. These results translate into a different prognosis for individual patients with "apocrine" carcinoma.

198 Beta-Catenin/WNT Signaling Pathway in Fibromatosis, Metaplastic Carcinomas and Phyllodes Tumours of the Breast

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Background: Wnt signalling pathway is known to play a critical role in carcinogenesis and in epithelial to mesenchymal transition. Upon Wnt activation, β-catenin (CTNNB1) is translocated from the membrane to the cytoplasm and nucleus, where it interacts with transcriptional activators. It has been demonstrated that fibromatoses harbour APC mutations and that metaplastic breast carcinomas may harbour CTNNB1 mutations, both leading to Wnt pathway activation. Given that β-catenin nuclear localisation constitutes a good marker of Wnt canonical pathway activation, we have investigated the distribution of β-catenin in spindle cell lesions of the breast and whether it could be employed in the differential diagnosis of these lesions.

Design: 52 metaplastic breast carcinomas (MBCs) (22 spindle cell carcinomas, 17 carcinomas with squamous metaplasia, 13 carcinomas with heterologous elements), eight fibromatosis and 23 phyllodes tumours (PTs), were retrieved from our institutions' archives. We performed immunohistochemistry using two commercially available anti-β-catenin antibodies on whole tissue sections. β-catenin nuclear, membranous and cytoplasmic expression was assessed in each tumour component using the Allred scoring system. Each component of a case with a score>2 was considered as positive.

Results: A good correlation between the 2 antibodies used in this study was observed (Spearman's rho 0.675, p<0.001). All fibromatoses analysed expressed nuclear β-catenin. 23% of MBCs displayed β-catenin nuclear expression. No association between the subtype of MBC and β-catenin nuclear expression was found. Membranous expression was lower in spindle cell carcinomas (mean score 4) than in carcinomas with squamous metaplasia and with heterologous elements (mean scores 8 and 7, respectively). In PTs, β-catenin nuclear staining was observed in both the epithelial and spindle cell components. Spindle cells of benign and malignant PTs displayed nuclear β-catenin expression in 94% and 57% of cases, respectively. Membranous expression was observed only in the epithelial component of all PTs.
Conclusions: β-catenin nuclear expression is a common feature in fibromatosis and in the stromal component of PTs, but may also be observed in MBCs. β-catenin nuclear expression should not be used as a single marker to differentiate fibromatosis from other spindle cell tumours of the breast.

199 Type and Grade of Lobular Neoplasia in Breast Core Biopsy: Does It Matter for the Clinician?

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Background: The recent introduction of E-cadherin as a marker for ductal versus lobular origin of breast neoplasia, has led to the recognition of new histologic entities. Previous studies investigating the implication of in situ lobular neoplasia diagnosis on core biopsy are limited by the lack of categorizing the lesions according to their grades and histologic features. We studied the diagnostic implication of different histologic types of in situ lobular neoplasia in breast core biopsy.
Design: We retrieved core biopsies with Atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS) from the hospital archives during 5-years period. In addition, we reviewed core biopsies diagnosed as solid type ductal carcinoma in situ (DCIS) with comedo-type necrosis. Cases were immunostained for E-cadherin and reclassified based on their E-cadherin expression and morphology. Corresponding excision biopsies were examined in all cases.
Results: Out of 126 cases diagnosed as solid type DCIS with comedo necrosis, E-cadherin was negative in 17/126 (13%) and reclassified as LCIS with necrosis (necrotic type). All core biopsies were classified into: 22/48 (46%) cases of ALH/LCIS classic type, 9/48 (19%) cases as LCIS, pleomorphic type and: 17/48 (35%) cases of LCIS with necrosis. On resection, ALH/LCIS group showed benign breast lesion in 8/22 (36%), ALH in 7/22 (32%), LCIS in 2/22 (9%), DCIS/LCIS in 3/22 (14%), and infiltrating lobular carcinoma (ILC)/LCIS in 2/22 (9%). In pleomorphic LCIS group; resection revealed benign breast lesion in 3/9 (33%), LCIS in 2/9 (22%), DCIS/LCIS in 3/9 (33%) and ILC in 1/9 (11%). In LCIS with necrosis group; resection demonstrated pleomorphic ILC in 5/17 (29%), DCIS/LCIS in 4/17 (24%) and LCIS with necrosis in 8/17 (47%).
Conclusions: Our study showed as association between the morphologic features of lobular neoplasia in the core biopsy and histologic findings of lumpectomy. The likelihood of finding more advanced lesions is lower in cores with ALH/LCIS compared with LCIS pleomorphic type and those with necrosis. Also, our study showed that LCIS with necrosis is an under-recognized entity in core biopsy. Although the current recommendation is to treat them similar to DCIS, further investigation of their long term outcome is warranted.

200 PTEN Loss of Expression Is Not Related with PTEN Promoter Hypermethylation in HER2-Positive Breast Carcinoma

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Background: PTEN expression is reduced in at least 50% of breast carcinomas (BC) in the absence of mutations. This underexpression can be related to epigenetic factors, such as gene promoter hypermethylation, which has been reported in several human neoplasms. The aim of our study was to analyze the expression of PTEN by immunohistochemistry (IHC) and PTEN methylation status by Methylated Specific PCR (MSP) analysis after discrimination of the pseudogene psiPTEN, in a series of HER2-positive BC.
Design: We evaluated in 210 HER2-positive (3+ in >30% cells by IHC or amplification by FISH/CISH) BC the PTEN expression (Dako) by IHC. DNA methylation patterns in the PTEN promoter were determined by MSP in 68 BC (34 PTEN-positive and 34 with PTEN loss). Briefly, 1 µg of genomic DNA from tumor samples was subjected to bisulfite modification (Epitec Bisulfite Kit; Qiagen). PCR was carried out using specific primers (methylated or unmethylated). Commercial genomic DNA was used as negative and positive controls, after treatment with CpG methyltransferase M.SssI (New England BioLabs). PCR products were analyzed using 2100 Bioanalyzer. The results were correlated with clinical-pathological parameters and the outcome.
Results: Patients' average age was 58 years (range 24-87 years) with a median follow-up of 73 months. Tumors were more frequently of high grade (94.8 %), with necrosis (51%) and lymphatic invasion (54.8 %). PTEN loss of expression (complete absence) was seen in 16.2% (34/210) tumors. PTEN was predominantly preserved in tumors of younger patients (p=0.003), with <2 cm in size (p=0.004), and a trend with lower grade (p=0.10), but without differences for presence of necrosis, vascular invasion or lymph-node status (p=ns). The molecular analysis revealed 98.3% samples without promoter hypermethylation, and only 1 tumor PTEN-positive showing partial methylation (1.7%). Patients with PTEN-positive tumors had better disease-free survival than those with PTEN loss (66.4% vs 50%; p=0.048), but the overall survival was not different (76% versus 69%; p=ns) (Kaplan-Meier; log rang test).
Conclusions: In our series of HER2-positive BC, 16.2% showed PTEN loss. The lack of association between absence of PTEN and promoter hypermethylation status suggests that in addition to mutations, another epigenetic factors such as protein degradation, may contribute to this alteration in this subtype of BC. Supported by grant FIS 06/1495.

201 Risk Factors for Non-Sentinel Lymph Node Metastasis in Breast Cancer Patients with Positive Sentinel Lymph Node

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Background: The role of complete axillary lymph node dissection (ALND) after identification of metastasis in sentinel lymph node (SLN) biopsy of woman with breast cancer has been questioned. Up to 60% of patients with positive SLN are found to have no other metastasis in non sentinel lymph node (NSLN). The aim of our study was to evaluate risk factors for NSLN metastasis in SLN-positive patients and to propose a mathematical model (nomogram) to predict the likelihood of finding additional positive nodes at ALND.
Design: We reviewed 326 cases of patients with breast cancer and positive-SLN divided into two groups according to the nodal involvement in the ALND: patients with all non-sentinel nodes negative for metastasis and patients with at least one positive NSLN. Pathological features of the primary tumor (tumor size, histological tumor type and grade, mitotic index, nuclear grade, lymphovascular invasion, estrogen and progesterone receptor status), and SLN (number of SLN, detection method of metastasis and size of SLN metastasis) were assessed. Data were submitted to univariate and multivariate logistic regression, followed by construction of a mathematical model (nomogram) to predict the presence of additional disease in the non-SLN of these patients.
Results: The univariate and multivariate analyses identified the following risk factors for involvement of NSLN with the respective p values: length of the largest SLN metastasis ($p < 0.001$, $p = 0.002$), number of positive SLN ($p = 0.006$, $p = 0.04$) and negative SLN ($p = 0.010$, $p = 0.004$), and lymphovascular invasion (LVI), ($p = 0.075$, $p = 0.085$). The nomogram was created using size of largest SLN metastasis, number of positive and negative SLN, and LVI. The nomogram was discriminating, with an area under the receiver operating characteristic (ROC) curve of 0.70.
Conclusions: Our data showed that the size of largest SLN metastasis, number of positive and negative SLN were predictive risk factors for metastatic involvement of NSLN in patients with positive-SLN. Our nomogram, similar to other models, may represent an additional tool to help physicians and patients who decide whether or not a complete ALND should be performed.

202 Detection of microRNA-21 Expression in Pregnancy-Associated Breast Cancer: A Possible Marker of Poor Prognosis

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Background: miRNAs are small fragments of non-coding RNAs that are involved in the regulation of gene expression. Recent studies have shown that specific microRNAs can act as key modulators of tumorigenesis. Specifically, miR-21 overexpression has been suggested to play an important role in the development and progression of breast cancer (BC). The existence of specific miRNAs in pregnancy-associated breast cancer (PABC) is not known. We evaluated the presence of miR-21 in cases of PABC, compared the expression with regular BC and correlated with known prognostic factors.
Design: A total of 35 patients, 25 PABC and 10 cases with similar clinicopathological features but not pregnancy associated were analyzed for miR-21 expression by RT-PCR. MicroRNAs surrounding miR-21 (miR-301, miR-10a and miR-633) were also evaluated to confirm the specificity of the results for the miR-21. We then analyzed protein expression by IHC for potentially targeted genes, including PTEN, Bcl-2 and PDCD4, and finally correlated the results with patient's clinico-pathological features.
Results: Patient's mean age was 35 yrs. Lymph node metastasis was present in 75% of cases. All tumors were infiltrating ductal carcinomas; 32 % were histologic grade 2 and 68% were grade 3. In the PABC cases compared with the control group, positive ER status was seen in 19% vs 37% of patients, 62.5% were negative for PR in all cases and 100% vs 66.7% were HER-2 negative. We found overexpression of miR-21 in all tumor samples compared with their own normal adjacent tissue. Overexpression was significantly correlated with high histological grade, but not with lymph node status or the presence of advanced disease. PTEN protein expression was negative in 80% of the cases, PDCD4 expression was cytoplasmic positive in 75% of the cases and 60% of tumors were negative for Bcl-2. PTEN negative expression was significantly correlated with increase of miR-21 (p=0.044).
Conclusions: Our results demonstrate important miR-21 overexpression in PABC. Changes in target genes such as PTEN and Bcl-2 support the concept that miR-21 may play an important oncogenic role in breast cancer in general. Expression of this miRNA may be a possible indicator of poor prognosis in breast cancer.

203 Positive Expression of Enhancer of Zeste Homolog 2 (EZH2) Predicts Shorter Overall Survival in Patients with Inflammatory Breast Cancer

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Background: Enhancer of Zeste homolog 2 (EZH2), a member of Polycomb Group (PcG) proteins, is a known repressor of gene transcription and has been reported to be implicated in stem cell regulation and cancer development and progression. We sought to determine the relationship between the expression of this candidate stem cell marker and the prognosis of patients with inflammatory breast cancer (IBC), a rare but the most lethal form of breast carcinoma.

Design: Tissue microarray samples from 85 surgically removed IBCs between September 1994 and August 2004 were analyzed. Patients' information, tumor characteristics and prognostic and predictive biomarkers were retrospectively reviewed. Immunhistochemical staining for EZH2 was performed using monoclonal antibody (clone 6A10, dilution 1:200, Novocastra). Nuclear staining in greater than10% of tumor cells was defined as positive. The relationships between overall survival (OS) and EZH2 expression, other pathologic parameters, and biomarkers were examined.
Results: The age of the patients ranged from 23 to 75 years (median: 49.5 years). Median follow-up time was 8.43 years. Fifty-two deaths occurred by the time of analysis. Median OS was 4.04 years (95% CI, 2.85, 10.2). EZH2 expression was found in 53 (64%) of the 83 IBCs, ER expression in 35 (42%), PR expression in 29 (35%), and HER2 overexpression and/or amplification in 32 (39%). Univariate analysis of OS calculated by the Kaplan-Meier method revealed that poorer OS was significantly associated with positive EZH2 expression (p=0.01) and negative ER status (p=0.006). The 5-year OS for patients with tumors expressing EZH2 was 32.1%, compared to 64.2% without EZH2 expression (log rank $P = 0.01$). There was no significant correlation between OS and histologic type, nuclear grade, lymphovascular invasion, nodal status, PR status, or HER2 status. In addition, EZH2 expression was more frequently seen in tumors with higher nuclear grade (p=0.03) and negative ER status (p<0.01).
Conclusions: IBC patients with positive EZH2 expression and negative ER status had a significantly poorer prognosis. Positive EZH2 expression was significantly associated with higher nuclear grade and negative ER status. These findings indicate that EZH2 may serve as a novel biomarker for predicting prognosis and may be a potential candidate for targeted therapy in patients with IBC.

204 Markers of Metastatic Breast Cancer: Correlations between GCDFP-15 or Mammaglobin Expression and Tumor Grade, Hormone Receptor, and HER2 Expression

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Background: Both the gross cystic disease fluid protein-15 (GCDFP-15) and mammaglobin represent excellent markers of carcinomas of breast origin that can be applied in the analysis of carcinomas presenting at metastatic sites, showing sensitivities of nearly 80% and 60%, respectively, with a combined sensitivity of almost 90%. However, there are few published studies examining the relationship of expression of these breast-restricted markers with breast cancer grade and expression of the biomarkers ER, PR, and HER2, which could have important implications in their use in certain clinicopathologic settings.
Design: A series of 334 breast cancers were tested on whole tissue sections for expression of GCDFP-15 (Leica/Novocastra, clone 23A3) mammaglobin (Zeta Corp, clone 31A5), estrogen receptor (Thermofisher, SP1), progesterone receptor (Dako PgR636), and HER2 (Thermofisher, SP3). Scoring was based on percentage of positive tumor cells: negative (0%), rare cells (<1%), focal (1-25%), variable (26-75%), uniform (>75%) except for HER2, which was scored as positive, negative, or equivocal. Tumors were graded using a modified Nottingham score (1 through 3). Chi-square and other statistical analyses were performed to determine the expression relationships among the different markers.
Results: As expected, there was a strong inverse correlation found between HER2 and ER expression (p< 0.0001), but also a strong positive correlation between GCDFP-15 and mammaglobin expression (p<0.0001). Statistically significant positive correlations were found between ER expression and expression of GCDFP-15 (p=0.007) and mammaglobin (p=0.012), but no relationship was found between expression of either of the breast-restricted markers and HER2 expression (p=0.10, GCDFP-15; p=.81, mammaglobin). Strong inverse correlations were found between Nottingham grade and both GCDFP-15 (p<0.0001) and mammaglobin expression (p=0.006).
Conclusions: While GCDFP-15 and mammaglobin are excellent markers of carcinomas of breast origin presenting at metastatic sites, caution should be used in their use in the context of high grade, ER-negative cancers given the strong correlations found between these biomakers and expression of both GCDFP-15 and mammaglobin.

205 Breast Carcinoma Following Treatment of Hodgkin Lymphoma: A Study of 31 Patients

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Background: Approximately 30% of women successfully treated with supra-diaphragmatic radiation for Hodgkin lymphoma (HL) develop breast cancer, placing them in a high-risk category. Whether and how the clinical and pathologic characteristics of breast cancer in this setting differ from those of sporadic breast cancer is essential to developing improved screening and treatment approaches in this unique group of breast cancer patients.
Design: This is a retrospective study of 31 patients with breast cancer that occurred after treatment for HL, whose breast cancers were pathologically confirmed in our department between 1991 and 2009. We studied clinical records, pathology reports and histologic slides to determine the mode of clinical presentation, pathologic features, AJCC stage and predictive marker profile of 38 cancers occurring in these 31 patients.
Results: Median age at breast cancer diagnosis was 43 years. Mode of clinical detection, known for 29 of 38 cancers, was: palpable mass = 13; mammogram = 13; MRI = 3. Most cancers occurred in the upper quadrants (81%) & were unicentric (97%), i.e. grew as a single mass. The majority of ***index*** cancers (26/31 = 84%) were invasive; 5/31 (16%) were DCIS-only at the time of diagnosis. AJCC stage for the index cancer was known for 23 patients with invasive carcinoma: Stage I = 11/23 (48%); Stage II = 9/23 (39%); Stage III = 1/23 (4%); Stage IV = 2/23 (9%). Seven patients (23%) developed contralateral carcinoma (3 synchronous; 4 metachronous), with 3 of the metachronous carcinomas occurring ≥ 10 years after the index cancer. Contralateral cancer was invasive in 4/7 (57%) & DCIS-only in the other 3. One contralateral DCIS was detected by MRI – alone; however, very few patients had MRI exams. Five of 26 (19%) index invasive cancers were negative for ER, PR & HER-2/*neu* ("triple-negative"); whereas, none of the 4 contralateral invasive cancers had this phenotype.
Conclusions: Except for occurring at a higher rate, at a lower median age and perhaps, more often in the upper quadrants, breast cancer in this high-risk context shares many features with sporadic breast cancer. These cancers do not appreciably differ with respect to rate of multicentricity, bilaterality, stage at presentation or prevalence of the "triple-negative" phenotype. Efforts to improve screening in these patients should focus on increasing the number of patients identified at the *in situ* stage of cancer. The pathologist's tasks are the same as for sporadic breast cancer – establishing AJCC stage and the cancer's predictive marker profile.

206 Amplification of the FGRF1 Gene and Its Relationship with Gene Expression and Clinical Significance in Breast Cancer

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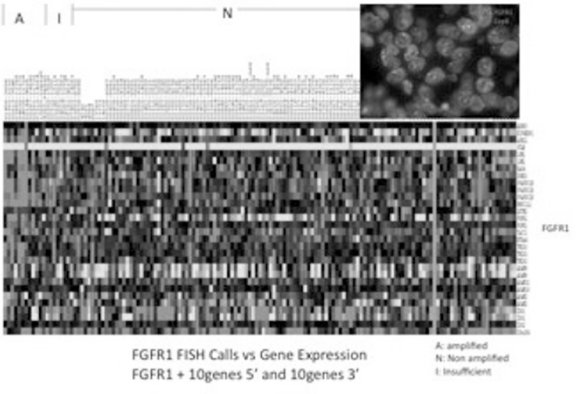
Background: Fibroblast receptor growth factor 1 (FGFR1) plays a pivotal role in breast development. The gene for FGFR1, located on chromosome 8p11.2, is amplified in 10-15% of invasive breast cancers (IBCs). The relationship between gene amplification and expression, and its clinical significance, are not well understood. The purpose of this study was to evaluate these relationships.
Design: A FISH assay was developed to measure FGFR1 gene copy number, based on a newly designed probe for chromosomal region 8p11, and a commercially available probe for the corresponding centromere. FISH was used to determine FGFR1 status on a previously constructed tissue microarray (TMA) containing 139 IBCs with available data on gene expression profiles (microarray analysis), standard clinical-pathological features (age, race, tumor type, grade, size, nodal status, ER, PgR, HER2), and patient survival. SPSS V13.0 software was used to evaluate the significance of relationships between gene copy number and other variables (student t-test, Kaplan-Meir curves, and Cox-Wilson regression).
Results: The FGFR1 gene was amplified in 9.4% (13/139) of IBCs. Gene expression was significantly increased (>2-fold) in amplified vs. non-amplified IBCs (84% vs. 2%, p≤0.05). Several other genes near FGFR1 in the region of chromosome 811p.2 (e.g. BAG4, PPAPDC1B, TM2D2) were also over-expressed. Polysomy of chromosome 8 was observed in 15% (21/139) of cases, but only 19% (4/21) showed elevated expression of FGFR1. There were significant (p=.05) correlations between amplification of FGFR1 and patient age (older), tumor histological grade (higher), ER/PgR status (positive), and invasive lobular subtype of IBC. In multivariate analyses patients with FGFR1 amplified IBCs showed significantly longer average survival than patients with non-amplified tumors (60 vs. 53 months, p=.029).
Conclusions: FGFR1 was amplified in 9.3% of IBCs (n=139). Gene amplification was strongly associated with elevated expression, and significantly longer patient survival.



207 DNA Damage-Associated Proteins in Triple Negative Breast Cancers

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Background: Triple negative (TG) and basal-like (BL) tumors show frequent concordant loss of Fhit and Wwox proteins encoded by chromosomal fragile loci, FRA3B and FRA16D, that are exquisitely sensitive to genotoxic stress. In this study, the activation status of other DNA damage response-associated proteins was also examined in TN/BL tumors.
Design: TMAs were constructed from 479 breast cancer blocks and immunostaining performed. In addition to Fhit and Wwox, expression of basal markers CK5/6 and EGFR, DNA damage response proteins BRCA-1, p53, pChk2 and γH2AX, Wwox-interacting proteins Ap2α, Ap2γ, and ErbB3/B4 were scored. Expression was considered positive for tumors with: >10% cytoplasmic CK5/6, membranous EGFR, nuclear Ap2α/γ; >50% of cells with nuclear γH2AX, >25% with nuclear pChk2; any membrane staining of ErbB3 or ErbB4. Cytoplasmic Fhit and Wwox staining was scored for intensity: no staining, highly reduced, reduced or strong expression. BRCA-1 nuclear expression was scored as: high (>90%), reduced or negative.
Results: TN and BL (CK5/6 or EGFR+) tumors showed reduced Fhit, Wwox, BRCA-1 and ErbB3 (TN tumors, p<0.001,p=0.001,p=0.017,p=0.012 respectively; BL tumors,

p=0.010,p=0.016, p=0.008,p=0.017 respectively) and more γH2AX, pChk2, p53, Ap2γ and ErbB4 expression (TN tumors, p=0.034,p=0.056, p<0.001, p<0.001, p=0.015 respectively; BL tumors, p=0.0182 p=0.030, p<0.001,p=0.001,p=0.028 respectively).
Conclusions: Absence of BRCA-1, with evidence of persistent activation of DNA damage response checkpoints in a large fraction of TN and BL tumors suggests that alteration or abrogation of signal pathways associated with cell cycle checkpoint control is characteristic of these tumors. Definition of specific DNA repair and checkpoint defects may provide new treatment targets.

208 Hepatocyte Growth Factor/Scatter Factor and Its Receptor c-Met Modulate E-Cadherin Expression in Matrix-Producing Breast Carcinomas

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Background: Hepatocyte growth factor (HGF)/ scatter factor (SF) is a cytokine known to regulate epithelial mesenchymal transition (EMT) and to stimulate proliferation, motility, angiogenesis and invasion in various cell types, including breast carcinomas. Its biologic signal is transmitted via the HGF receptor c-Met. E-cadherin plays an important role in the maintenance of epithelial cell-cell adhesions. HGF phosphorylates β-catenin and modulates the E-cadherin complex mediated cell-cell adhesions by β-catenin dissociation from E-cadherin, leading to distribution of E-cadherin from a functional to a nonfunctional compartment in the cell. Matrix-producing breast carcinoma (MBC) show a mixed epithelial and mesenchymal chondromyxoid matrix with features reminiscent of EMT and therefore are a potential in vivo model for EMT. In this study we investigated the correlation of HGF/SF, c-Met and E-Cadherin expression in both tumor components.
Design: Archival paraffin embedded material of twelve matrix-producing breast carcinomas as defined by the 2003 WHO classification were examined by IHC for the expression and localization of HGF/SF, c-Met and E-cadherin.
Results: Overall, 92% of study cases revealed cytoplasmic HGF and c-Met expression. The HGF immunoreactivity was mainly located at the interface of cellular areas with chondroid elements and adjacent to conventional invasive ductal carcinoma. C-Met was equally expressed in both tumor components. For E-cadherin, 83% of cases showed membranous staining in the conventional ductal carcinoma, reduced expression at the interface, and complete loss of expression in the metaplastic component. Two cases were completely negative for E-cadherin.
Conclusions: The MBC included in this study showed a high frequency of c-Met receptor protein activation, which is the only known receptor for HGF/SF. Our study revealed an inverse correlation between HGF expression and membranous E-cadherin immunoreactivity, supporting HGF's role in modulation of E-cadherin. Of interest was the reduced E-cadherin expression in the chondroid metaplastic component. Loss of E-cadherin expression leads to less cell-cell adhesions and increased motility of cells (cell 'scattering'), both of which are features of epithelial-mesenchymal transition (EMT). Our findings therefore suggest that the tumor cells of the metaplastic chondroid component acquire a mesenchymal phenotype by undergoing EMT. This might contribute to the high metastatic potential and the known aggressive clinical behavior of MBC.

209 Morphologic Features of Breast Carcinoma Are Associated with False Negative Screening by Axillary Ultrasound

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Background: Axillary ultrasound (AUS) and ultrasound-guided lymph node biopsy (LNB) are important breast cancer staging adjuncts, obviating the need for sentinel lymph node biopsy (SLNB) in many cases. Patients with normal findings on AUS/LNB still undergo SLNB to ensure adequate screening. Given the complexity of this screening algorithm, it is necessary to identify patient subsets that are less likely to benefit from AUS screening.
Design: 461 patients who underwent AUS screening (2004-2008) were stratified into 4 groups: true positive (TP), false positive (FP), true negative (TN), and false negative (FN) according to final nodal status determined by ultrasound-guided axillary node biopsy, follow-up SLNB or axillary dissection. Histology of resected primary breast lesions from a subset (236 consecutive cases; 2004-2006) of these patients was reviewed to determine whether lymphoplasmacytic infiltrate, tumor necrosis, micropapillary features or lobular-like growth pattern were associated with failure of AUS screening.
Results: In total, 135 patients were node positive and 326 were node negative. AUS/LNB accurately predicted final nodal status in 75% of cases (348/461; sensitivity 0.50, specificity 0.86, PPV 0.60, NPV 0.81). Micropapillary features were present in 38 cases (range: 5% to 100% of tumor volume) and were more prevalent in FN cases than in TN cases (26.3% vs. 12.7%; p = 0.047). Lobular-like growth pattern was identified in 55 cases (range:10-100% of tumor volume) and was more prevalent in FN cases, compared to TN cases (39.5% vs. 22.5%; p = 0.04). Lymphoplasmacytic infiltrate and tumor necrosis did not vary significantly between the four examined groups.
Conclusions: As a screening method, AUS/LNB has relatively low sensitivity. Identifing factors that contribute to the high FN rate may improve this method's sensitivity. This study identifies micropapillary features and lobular-like growth pattern as two morphologic characteristics of the index breast carcinoma that are more likely associated with FN screening. Patients whose primary lesion exhibits these features on biopsy may benefit from proceeding directly to SLNB in order to eliminate the costs of unnecessary procedures. Larger studies are warrented to further validate these results.

210 Comparative Analysis of Ki67 Expression and Its Association with Axillary Nodal Metastasis in Patients with Triple Negative Versus Non-Triple Negative Breast Carcinomas

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Background: Triple negative (TN) breast carcinoma characterized by negativity for estrogen receptor (ER), progesterone receptor (PR) and Her2, is associated with shorter survival and a higher recurrence rate. Ki67 expression, a proliferation marker, is considered as a prognostic marker for patients with breast cancer. The aims of our study are to compare Ki67 expression in TN and non-TN breast carcinomas and to investigate an association between the Ki67 expression and axillary nodal metastasis in these tumors.
Design: Clinical characteristics and immunohistochemical tumor profiles were analyzed in 214 patients with high-grade invasive ductal carcinomas including 81 TN tumors and 133 non-TN tumors. Of the 133 non-TN tumors, 18 (13.5%) were ER-PR-Her2+, 18 (13.5%) were ER+PR±Her2+, and 97 (73%) were ER+PR± Her2-. The expression of Ki67 was compared between the TN and non-TN groups by one-way ANOVA with Turkey's post hoc test. In addition, a comparative analysis of the Ki67 expression in the TN tumors with nodal metastasis (n=32, 40%) versus the non-TN tumors with nodal metastasis (n=63, 47%) was conducted with unpaired t test.
Results: The TN tumors were larger than the non-TN tumors in size (3.18cm ± 0.45 vs. 2.31cm ± 0.21, p<0.05). The expression of Ki67 in the TN group was significantly higher than that in the non-TN group (63.7% ± 3.58 vs. 30.3% ± 2.37, p<0.0001). Of note, Ki67 expression in the TN tumors was significantly increased compared to the non-TN tumors that were ER+PR±Her2- (p<0.0001) or ER+PR±Her2+ (p<0.001), but there was no significant difference of the Ki67 expression between the TN tumors and the non-TN tumors that were ER-PR-Her2+. In addition, Ki67 expression in the TN tumors with nodal metastasis was significantly higher than that in the non-TN tumors with nodal metastasis (64.0% ± 6.02 vs. 31.9% ± 3.40, p<0.0001).
Conclusions: Our results indicate that TN breast tumors are associated with a significantly higher expression of Ki67 compared to non-TN tumors, which may contribute to the poorer prognosis in TN tumors. Hormonal receptor negativity rather than Her2 negativity appears to be the determining factor in significantly increased Ki67 expression in TN tumors. Furthermore, high Ki67 expression is more likely to be associated with axillary nodal metastasis in patients with TN tumors vs. non-TN tumors.

211 Contralateral Prophylactic Sentinel Lymph Node Biopsy (CP-SLNB) for Breast Carcinoma: Too Much Dissection for Too Little Gain?

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Background: The potential value of performing contralateral prophylactic sentinel lymph node biopsy (CP-SLNB) has been incompletely studied. In theory if an invasive carcinoma were to be found in a contralateral prophylactic mastectomy (CPM), a CP-SLBN would obviate the need for contralateral axillary dissection. However the chance of finding contralateral carcinoma is generally believed to be low. To address this question more fully we reviewed our most recent 6 year-experience with CP-SLNB.
Design: Our institution's surgical pathology files were queried for all specimens coded as "mastectomy". An intentionally "wide net" was cast in order to fully maximize catchment. This voluminous list was then culled by hand to include cases of CPM for: (1) patients undergoing total mastectomy for recently diagnosed ipsilateral carcinoma, (2) patients reporting a history of ipsilateral carcinoma previously treated by mastectomy and (3) patients undergoing bilateral mastectomies without a diagnosis of breast carcinoma (this included women with atypia, lobular neoplasia, positive family history, genetic mutations, and one woman with ovarian carcinoma). Excluded were contralateral mastectomies performed for a suspicious lesion or biopsy proven carcinoma. Data were abstracted from anonymized surgical pathology reports to include age, laterality, ipsilateral primary cancer histology, contralateral histology and CP-SLNB results, as well as any available clinical history.
Results: During 2004-2009 there were 226 patients undergoing CPM and there was increasing utilization of CPM over this interval (i.e. 10, 29, 33, 38, 50 & 66 per annum, respectively). Patients were all women aged 29-78 yr. (mean 48.8). Of these 226, 176 (78%) also underwent CP-SLNB. A total of 391 CP-SLN were harvested (mean 2.22). There were isolated CK(+) cells in a single lymph node from one patient (2 additional intramammary LN were negative). This 37-year-old woman had ipsilateral DCIS and pleomorphic LCIS and had recently had tested BRCA positive. The PCM showed only DCIS after extensive sectioning.
Conclusions: The positive yield of CP-SLNB is extremely low. In this study there was only a 0.57% (1/176) incidence of N0[i+] disease and not even a single N1 case. Even considering the highest risk individuals (i.e. BRCA positive with ipsilateral carcinoma), it may soon become standard of care to forgo CP-SLNB in most CPM.

212 Primary Breast Malignant Fibrous Histiocytoma (MFH): A Clinicopathologic Review of 19 Cases with Two New Variants

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Background: Primary breast MFH is rare and can pose a diagnostic challenge. We reviewed our experience with this entity.
Design: Cases coded as "MFH," "myxofibrosarcoma" or "pleomorphic sarcoma NOS" were culled from our registries. Cases meeting WHO morphologic and immunohistiochemical criteria for MFH variants were included; FNCLCC grading scheme was applied. Recorded histologic features included inflammation, vascular pattern, stromal appearance, mitotic activity, necrosis, cell, nuclear and nucleolar morphology, and growth pattern. Clinical and follow-up data were obtained. Only

primary cases in breast were included. Eight cases were excluded due to better diagnosis as another tumor or insufficient material.
Results: Nineteen patients with primary breast MFH included one male and 15 females, ranging in age from 15 to 86 years (mean, 59). Tumor size ranged from 1.3 cm to 15 cm (mean, 6.3). Most patients received wide local excision with or without adjuvant therapy. Five patients with an older mean age (70 years) had distant metastases and died of disease within from one to 19 months (mean, 6). Cases were MFH storiform/pleomorphic (10/19) and giant-cell (1/19) subtypes and myxofibrosarcoma (6/19). Other histologic features were entrapped breast epithelium (14/19), hemangiopericytoma-like vessels (5/19), and prominent eosinophilic nucleoli (5/19). Unique lymphocyte-rich and pleomorphic hyalinizing angiectatic tumor (PHAT)-like variations were observed. Lymphocyte-rich variant showed pleomorphic spindle cells and prominent intratumoral lymphocytes, many in aggregate. PHAT-like variant showed large ectatic vessels, hemosiderin, pleomorophism, but with brisk mitotic activity. Cases were negative for pancytokeratins, S100, CD34 and desmin.
Conclusions: Primary breast MFH mostly affects middle-aged women. Distant metastases and older patient age appear to be associated with poor outcome. Storiform/pleomorphic and myxofibrosarcoma are the most common subtypes. Entrapped breast epithelium should not be misinterpreted as the epithelial component of a biphasic tumor. We present novel lymphocyte-rich and PHAT-like tumors. Careful attention to histology and immunohistochemistry of primary breast MFH can prevent misdiagnosis.

213 Do True Mixed Invasive Ductal and Lobular Carcinomas of the Breast Exist?

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Background: Invasive breast carcinomas can be divided into invasive ductal (IDC) and invasive lobular (ILC) type, based on histologic and cytologic features. There is a small group of invasive breast tumors showing features of both ductal and lobular neoplasia. Pathologists frequently classify these as invasive mammary carcinomas with mixed ductal and lobular features. Are these tumors truly mixed or are they ductal or lobular carcinomas with areas mimicking dual differentiation? A study of this subset of tumors may give us insight as to the frequency of true mixed tumors, and allow one to better predict risk of bilaterality, and route of metastases in these patients.
Design: With a computer search we identified 51 cases of invasive mammary carcinoma with mixed ductal and lobular features based on histomorphology from 2005-2009 (10 needle biopsies and 41 lumpectomies or mastectomies). E-cadherin immunostain was performed in all cases on sections that showed dual histology. Both H&E and immunostains were reviewed together, and presence or absence of E-cadherin was assessed in both components.
Results: The E-cadherin stain was diffusely and strongly positive in 35 cases and these were re-classified as IDC. In 8 cases, all tumor cells were negative for E-cadherin and they were re-classified as ILC. In the remaining 8 cases, the tumors had both positive and negative components. In five of these 8 cases, E-cadherin positive and negative tumor cells were present in the same areas, and these cases were classified as true mixed tumors. In two cases, two separate tumor masses were identified in the same breast; one was positive and the other negative, and two separate diagnoses were rendered; one of these two cases had E-cadherin negative tumor cells metastatic to the lymph node. In one case, all tumor cells in the breast mass were positive for E-cadherin, but the metastases in the axilla showed both E-cadherin positive and negative tumor cells, suggesting a true mixed neoplasm in which the lobular component may not have been sampled.
Conclusions: Most tumors with mixed ductal and lobular features on H&E are pure IDC (68.6%), with few being pure ILC (15.7%). True mixed invasive mammary carcinomas with ductal and lobular immunophenotypes (E-cadherin positive cells and E-cadherin negative cells in the same tumor mass) do exist, but they make up only a small fraction of the cases (9.8%).

214 The Prognostic Benefit of the Histologic Grade for Breast Cancer after Integration into the TNM

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Background: Tumor grade is a prognostic factor for breast cancer, yet it is separately reported and not integrated into staging systems. We hypothesize that incorporation of histologic grade into the TNM would provide added stratification for the staging system.
Design: Data were obtained from NCI's SEER program for years 1990-2000. There were 140,714 cases of breast cancer. Using novel machine learning algorithms based on a censoring cluster analysis, 10 year disease specific overall survival and hazard rates were calculated for certain combinations of tumor size (T), nodal status (N), metastatic status (M), grade (G), and estrogen receptor status (ER). T and N categories were classified according to the AJCC. Survival curves were compared for statistical significance by the log-rank test.
Results: The 10-year relative survival rate for patients with T2, N2, M0 breast cancer is 58%. Integrating ER status and grade into the TNM produces a disease specific survival for ER+ tumors of 73%, 65%, and 53% respectively for grade 1, 2, and 3 tumors. Similarly, for tumors T3, N2, M0, the ten-year relative survival is 44%. Incorporating ER status and grade into the survival analysis produces a disease specific survival for ER+ tumors of 57%, 50%, and 44% respectively for grade 1, 2, and 3 tumors. All differences in rates were statistically significant. In nearly every combination of T, N, and ER, histological grade proved to be a significant prognostic factor. When integrated into the TNM, grades 1, 2, and 3 progressively and significantly reduced the 10 year overall survival and altered the hazard rates regardless of tumor size, nodal status, and ER status. Even with N3 disease, grade still remained a significant prognostic factor.
Conclusions: When integrated into the TNM, grade becomes a significant prognostic factor regardless of tumor size, nodal status, and ER status. The prognostic benefit of grade, and perhaps other prognostic factors, is best demonstrated after integration into the TNM.

215 Estrogen Receptor-beta Expression in Normal Breast Tissue in Relation to Benign Breast Disease Category and Breast Cancer Risk: Results from the Nurses' Health Study

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Background: Estrogen receptor alpha (ER-α) and estrogen receptor beta (ER-β) are expressed in normal breast tissue, benign breast lesions and breast cancers but their relative importance in tumor progression is poorly-defined. We investigated the relation between ER-α and ER-β expression, benign breast disease (BBD) and breast cancer risk.
Design: Using a nested case-control design, we examined ER-α and ER-β expression in normal terminal duct lobular units (TDLUs) in relation to category of BBD and breast cancer risk among women in the Nurses' Health Study. We constructed tissue microarrays (TMAs) of normal TDLUs from benign breast biopsies and immunostained TMA sections for ER-α and ER-β. 246 women had normal TDLUs in the TMAs and evaluable ER-α and ER-β; 59 subsequently developed breast cancer and 187 did not. Epithelial cells in normal TDLUs were scored for ER-α and ER-β with positivity considered as >10% and >30% of nuclei staining, respectively.
Results: ER-α and ER-β expression in normal TDLUs in relation to category of BBD is shown in the Table. Women whose TDLUs were ERα-/ERβ+ more often had non-proliferative than proliferative lesions with or without atypia than those with other combinations of ERα/ERβ staining (p=0.007). In addition, absence of ER-β expression in normal TDLUs was twice as frequent among women who developed breast cancer (7.9%) than those who did not (4%), though this difference was not statistically significant (p=0.17).

Table: ERα and ERβ status in normal TDLU in controls with BBD

ERα/ERβ status	Non proliferative disease (%)	Proliferative disease without atypia (%)	Atypical hyperplasia (%)
ERα- ERβ-	2 (4.2)	0 (0)	0 (0)
ERα- ERβ+	9 (18.8)	7 (7.2)	1 (2.4)
ERα+ ERβ-	0 (0)	6 (6.2)	1 (2.4)
ERα+ ERβ+	37 (77.1)	84 (86.6)	40 (95.2)

Conclusions: Our results suggest that ER-β expression in normal TDLUs in the absence of ER-α expression is associated with the contemporaneous presence of BBD with a low level of risk of subsequent breast cancer (i.e. non-proliferative lesions). Further, these data suggest that absence of ER-β expression in normal TDLUs is more common in women who develop breast cancer than in those who do not. This lends further support to the notion that ER-β, particularly in the absence of ER-α expression, may result in an anti-proliferative phenotype and reduced risk of breast cancer.

216 Distinction of Pleomorphic from Classical Lobular Carcinoma In Situ: Proposition for a Minimum Diagnostic Criteria Based on Morphology

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Background: Recent data have shown no consistent immunophenotypic or genetic differences between pleomorphic LCIS (PLCIS) and classical LCIS (CLCIS), yet different management strategies are emerging following their distinction. Further, consensus criteria aiding distinction of PLCIS from CLCIS are lacking. In this study, we aimed to compare the morphologic features of PLCIS and CLCIS to determine if differences exist which could assist routine classification.
Design: 49 lesions {23 PLCIS and 26 classical lobular neoplasia (CLN)} in 45 women (4 had PLCIS and CLCIS) diagnosed in consultation from Jan 2005 to September 2009 were reviewed. Parameters examined were cellular dyscohesion, nuclear diameter, nuclear size variation, nucleolar presence and prominence (easily visible at x10 objective), number of mitoses per 10 high power fields (HPF), presence of necrosis and nuclear features of associated invasive carcinoma.
Results: All PLCIS lesions showed acinar distension by dyscohesive tumour cells with nuclear diameters ranging from 2-6x that of a small lymphocyte, with the following distribution: <2.5x (n=1), 2.5-<3x (n=8), 3-<4x (n=8), 4x and above (n=6). A 2-3 fold nuclear size variation was noted in all lesions of PLCIS. Nucleoli were observed in every PLCIS lesion; and were prominent in 15/23 lesions. Mitotic activity was detected in 20/23 (87%) PLCIS lesions, with an average mitotic index of 2.3/10 HPF (range 1-7). Tumour necrosis was present in 61% PLCIS lesions. 7 PLCIS lesions showed adjacent invasive lobular carcinoma; in six, both invasive and in situ tumour cells showed identical nuclear morphology whereas in one, invasive tumour cells showed significantly smaller nuclei (2x that of lymphocyte) than the in situ tumour cells (4x that of lymphocyte). In contrast, all CLN lesions exhibited nuclear diameters 1-2x that of a small lymphocyte and between 1-2x nuclear size variation (2x variation in some cells noted in 3 CLN lesions). Nucleoli (none prominent) were seen in 13 CLN lesions. None of the CLN lesions showed necrosis and two disclosed mitotic activity (1 mitosis/10 HPF in each).
Conclusions: PLCIS can reliably be distinguished from CLCIS on morphologic grounds, with parameters such as nuclear size and variation, presence of necrosis and mitotic activity being most helpful. We propose that LCIS composed of dyscohesive cells with at least 2x nuclear size variation and nuclear diameters greater than 2x that of a small lymphocyte as meeting the minimum criteria for a designation of 'pleomorphic' LCIS.

217 Mammary Adenomyoepitheliomas May Feature Basal/Spindle Cells with an Uncommitted Epithelial Phenotype: Biologic and Diagnostic Implications

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Background: Mammary adenomyoepithelioma (AME) is a rare tumour composed of luminal cells and prominent basally positioned polygonal-to-spindle myoepithelial cells, yet the "myoepithelial" nature of the latter cell population remains debatable given their conflicting expression for myoepithelial markers. We aimed to determine the phenotypic characteristics of AME, with particular emphasis on the nature of the so-called myoepithelial cells.

Design: A total of 19 AMEs in 18 patients diagnosed in consultation from January 2004 to September 2009 were reviewed. Immunohistochemistry for p63 (n=16), smooth muscle actin (SMA) (n=17), smooth muscle myosin (SMM) (n=15), CK5/6 (n=16) and CK14 (n=13) was performed using the biotin avidin method and reactivity for each marker was semi-quantitatively assessed. Positive internal controls were available. Focal positivity referred to <25% tumour cells stained.

Results: All patients, aged 40-82 years, presented with clinically or radiologically detected nodular masses, ranging in size from 4 to 60mm. All AMEs showed compact adenotic growth patterns with frequent (74%) central sclerosis and/or infarction.12 AMEs disclosed a focal spindle cell component merging in areas with the basal polygonal cells. On immunohistochemistry, basal/spindle cells of AMEs were commonly reactive for p63 (75%), SMA (94.1%), SMM (73%), CK5/6 (93.8%) and CK14 (100%), although the proportion of cells stained with each marker was highly variable (<5% to 100%). A significant proportion of AMEs contained basal/spindle cells that were either negative or only focally positive for the markers: p63 (37.5%), SMA (29.4%), SMM (60%), CK5/6 (43.8%) and CK14 (23.1%). Notably, 5 AMEs featured CK5/6 and/or CK14 positive basal/spindle cells which were both SMA and SMM negative; these cells occupied more than 50% of the entire basal/spindle cell population in 4 cases and were also p63 negative in 3 cases. In contrast, luminal cells of AMEs never stained with p63, SMA and SMM, but were frequently, albeit variably, positive for CK5/6 (93.8%) and CK14 (100%).

Conclusions: Mammary AMEs may prominently feature basal/spindle cells with an uncommitted epithelial phenotype characterized by basal keratin expression and absence of a myoid signature, the recognition of which may prevent misinterpretation of AME as malignant, particularly on needle core biopsy. While diagnostically useful, CK5/6 and CK14 are by no means specific surrogates for a myoepithelial phenotype.

218 Routine Excision of Core Biopsy Diagnosed Flat Epithelial Atypia Frequently Reveals ADH and Lobular Neoplasia: Potential Implications for Management

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Background: Flat epithelial atypia (FEA) is one of the earliest recognizable, non-obligate precursors of low grade breast cancer, of uncertain natural history. The management of FEA found on core needle biopsy (CNB) remains debatable, as limited data depict malignancy in 0-30% of cases excised. We aimed to determine the significance of FEA on CNB by studying the frequency of upgrade to ADH, lobular neoplasia (LN) and malignancy upon excision.

Design: Pathology and imaging data were reviewed for all cases of CNB-detected FEA between May 2005 and December 2008. CNB cases of FEA associated with ADH, LN or malignancy were excluded. Excision of FEA on CNB was routinely recommended. Statistical analysis (Fisher's exact test / t-test) was performed; differences with $p<0.05$ were deemed significant.

Results: Among 2657 CNBs, 28 (1.1%) cases of FEA were identified in 26 women with a mean age of 51 years. A mean of 12 cores of tissue were obtained per case, using 11 or 14 gauge biopsy needles, with at least 3 H&E sections examined per core. Indications for CNB included calcifications (n=23), density (n=1) and masses (n=4). All calcifications were of indeterminate nature. 22/28 (78.6%) cases were excised, all within 3 months of diagnosis, where none showed DCIS or invasive carcinoma. However, 45.5% of excision biopsies revealed breast lesions at significant risk of subsequent cancer: ADH (n=8), ADH+ALH (n=1) and classical LCIS (n=1). There were no statistically significant differences in patient age, laterality, biopsy needle gauge (11 vs 14) or number of cores obtained, between the group with (n=10) and the group without (n=12) ADH/LN on excision. Radiologic-pathologic concordance was achieved in all but 2 cases (a radiographic mass and a retroareolar density), where excision disclosed fibrocystic change with ADH, and an intraductal papilloma with adjacent ADH, respectively. Six patients who declined surgery (only 1 was biopsied for a mass, where histology showed fibroadenoma with adjacent FEA) have not presented with malignancy, 10 to 37 months post CNB diagnosis.

Conclusions: Despite no malignancy, routine excision of CNB diagnosed FEA frequently revealed lesions at moderate-to-high risk of breast cancer (ADH/LN), a finding hitherto unaddressed. Excision biopsy of CNB-detected FEA may be of value in identifying patients with ADH/LN who would benefit from appropriate follow-up and counseling, and possible chemopreventive therapy.

219 Prognostic Markers and Long-Term Outcomes in Ductal Carcinoma In Situ of the Breast Treated with Excision Alone

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Background: With increases in breast cancer screening, the number of cases of Ductal Carcinoma in Situ (DCIS) has risen dramatically. However, there remains no definite way to predict recurrence of DCIS. This study analyzed the significance of biological markers and tumor subtypes in predicting recurrence in a large series of DCIS patients with long term follow-up treated with local breast conservation surgery alone.

Design: Clinical and pathologic data was analyzed from 211 patients who underwent local excision alone (with negative margins) for DCIS diagnosed between 1983 and 2002. Using local disease recurrence as an endpoint, the authors sought to determine the prognistic significance of several histopathologic characteristics (tumor size, necrosis, and subtype) and biologic markers (estrogen receptor [ER], progesterone receptor [PR], and Her-2/neu.)

Results: With a median follow up of 122 months (max 294 months), 73 recurrences occurred with a median follow up of 147 months and a median month to recurrence of 232. Recurrences occurred between 9 and 294 months after initial diagnosis. In a multivariate analysis tumor size and Her2 positivity (3+) were found to be significantly associated with tumor recurrence (95% CI, $p=0.005$ and $p=0.012$). Necrosis and nuclear grade were not found to be significantly related to time to disease recurrence. Tumor pathologic characteristics were not found to be significantly related to time to disease recurrence. 59 out of the 73 recurrences were DCIS. 12 out of the 73 recurrences were invasive. None of the subtypes or biologic markers (ER or PR) was found to be a significant predictor of invasive versus noninvasive recurrence.

Conclusions: The current results suggest that over the long-term, larger tumor size and Her-2 neu status are significantly correlated with time to recurrence in patients treated by surgery alone. Using traditional logistic analysis, no significant correlation was found between tumor pathologic characteristics and recurrence.

220 Infiltrative Epitheliosis of the Breast: Clinicopathological Review of Seven Cases of "Pseudoinfiltrative Epitheliosis"

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Background: Infiltrative epitheliosis is a distinct breast lesion characterized by a pseudoinfiltrative proliferation of small cords of epithelial and/or often myoepithelial cells emanating from ducts with various patterns of epithelial proliferation into adjacent stroma. It may be associated with complex sclerosing lesions and it has been included in the spectrum of radial scar, despite its distinctive features. It is important to recognize this entity since it may be mistaken for invasive carcinoma particulary on core biopsies. A modified term - "pseudo-infiltrative epitheliosis" (PE) - is proposed to emphasize its benign nature and to avoid unnecessary additional surgery.

Design: Seven cases were retrieved from our institutional database in a two-year period (1/2007 to 9/2009) that included PE in the final diagnosis. Five of the cases were pulled from one of the author's personal consultation files. All H&E and immunohistochemical slides (including CK903, CK5/6, p63, calponin), and available clinical and radiological information were reviewed.

Results: All patients were female, with a mean age of 44.7 years (range: 27-61). Five of the specimens were lumpectomies, one was a mastectomy, and one case was diagnosed on mammotome biopsy. Three patients presented with a mass lesion, one patient had an area of enhancement on MRI, and in two patients PE was the secondary finding in a mastectomy performed for a known malignancy. Two consultation cases had been diagnosed as malignant by the primary pathologist and were determined to be benign PE on review. One case was submitted for consultation with a diagnosis of extensive papillomatosis and was found to have areas of PE admixed with infiltrating ductal carcinoma.

Conclusions: The pseudo-infiltrative pattern of epithelial and myoepithelial cells in PE mimics an invasive process, and may be easily misdiagnosed especially on smaller biopsy specimens. Confirmation of persistent epithelial and myoepithelial cell layers or pure myoepithelial cells on immunostains in association with papillomas or low risk epithelial proliferation rather than higher grade ductal intraepithelial neoplasia are helpful in supporting a benign process. However, the presence of PE does not rule out carcinoma as the two were intermixed in one of the cases reviewed. The term infiltrative epitheliosis may be confusing and may provoke unnecessary anxiety in patients and clinicians. Therefore, we propose using the term pseudo-infiltrative epitheliosis for this benign entity.

221 Correlation of Apocrine DCIS on Core Needle Biopsy with Excision

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Background: In situ apocrine proliferation in breast encompasses benign apocrine metaplasia, atypical apocrine metaplasia, atypical apocrine proliferation and overt apocrine ductal carcinoma in situ (DCIS). The rarity of apocrine DCIS and its unusual histologic features result in diagnostic difficulty in some cases, especially in core needle biopsy (CNB). To better understand its biological behavior, this study aims to examine aprocrine DCIS and borderline apocrine lesions diagnosed on CNB, and correlate histologic and radiological characteristics with excision outcome and follow-up.

Design: Breast cases coded as atypical aprocrine proliferation or aprocrine DCIS between 1999 and March 2009 were identified in our departmental computer database. Only cases with both CNB and excision reviewed and documented in our system were the subjects of this study, which included 27 apocrine DCIS and 9 apocrine proliferation bordering on or suspicious for DCIS diagnosed on CNB. Radiological characteristics including the size and nature of the target (mass vs. calcifications), and histologic features such as nuclear grade and presence or absence of necrosis on core biopsy were recorded and correlated with final diagnosis on excision and follow-up when appropriate.

Results: Of the 27 cases of apocrine DCIS diagnosed on CNB, 5 (19%) had invasive carcinoma on excision, including 1 microinvasion and 4 invasive ductal carcinoma ranging from 2 to 8 mm. The upgrade on excision did not correlate significantly with nuclear grade or necrosis on CNB, nor the nature of the radiological target ($p>0.05$, Fisher's exact test). The presence of invasion on excision inversely correlated with radiological size ($p=0.02$, student t-test) (average size: with no upgrade, 4.6 cm, n=16; with upgrade, 1.8 cm, n=4). Of the 9 cases of atypical apocrine proliferation bordering on or suspicious for DCIC on CNB, 7 cases had the diagnosis of DCIS on excision,

and 2 had no residual atypia. Follow-up on a total of 30 cases (range: 10 to 67 months; median: 22 months) revealed one DCIS patient with recurrence of apocrine DCIS at 41 months.

Conclusions: Apocrine DCIS is an uncommon type of in situ carcinoma in breast. The upgrade rate to invasion on excision with a CNB diagnosis of apocrine DCIS is similar to the previously reported rate for DCIS. The observed inverse correlation of lesional size and upgrade on excision suggests that size may not be a reliable parameter in predicting invasion in apocrine DCIS diagnosed on core biopsy. The lack of statistical significance of other parameters needs to be confirmed by a larger cohort.

222 Correlation of COX-2 Expression with Estrogen Receptor Status in DCIS

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Background: Ductal carcinoma in situ (DCIS) continues to represent a growing proportion of patients diagnosed with mammary neoplasia in recent years. While DCIS is considered a major precursor to invasive carcinoma, the progression rates are believed to be variable and potentially related to multiple morphological and molecular markers. Our objective was to classify DCIS according to ER/ Her 2 Neu profile and the expression of COX-2 as a marker likely to signify higher progression potential.

Design: We identified 108 consecutive patients with DCIS on whom clinicopathological data and ER status were available. Immunohistochemical staining for Her 2 Neu and COX 2 was performed using standard procedures. ER was considered positive if > 10% of the nuclei were stained while > 30% of DCIS cells showing complete membrane staining was required for Her-2/neu positivity. The resulting four DCIS categories (ER+, Her2/neu-; ER+,HER2/neu+; ER-,Her2/NEU-; ER-, Her2/NEU+), were correlated with the linear model for COX2 expression levels (expressed as percentages) and with patients age, race and with DCIS nuclear grade. All data was analyzed using the R statistical environment.

Results: Of the 108 DCIS cases, 40 were ER+, Her 2/neu-; 5 were ER+, Her2/neu +; 8 were ER-, Her2/neu + and 2 were ER-, HER-2/neu-. Increased expression of COX-2 was seen in the ER -, Her2/neu + group when compared to the ER+, Her2/neu + group (p=**0.004**) and to the ER+, Her2/neu – group (p=**0.0002**). The small number of cases in the ER-, Her2/neu- group precluded the inclusion of this category in the statistical analysis. COX-2 expression did not correlate with patient age, race or DCIS nuclear grade.

Conclusions: Our data suggest that the morphologic heterogeneity of DCIS appeared to be mirrored on the ER, Her2neu status and COX-2 expression levels. The study indicates statistically significant correlation between COX-2 expression and non-hormonally driven DCIS as determined by Estrogen receptor negative status.

223 Glucose Transporter Glut-1 Expression Correlates with Basal-Like Breast Cancer

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Background: Basal-like breast cancer, i.e., ER-negative, PR-negative, Her-2-negative, and basal cytokeratin (CK5/6 and or CK14)-positive, is a high risk breast cancer that lacks the benefit of specific therapy. Glut-1 is a facilitative glucose transporter that is expressed in normal tissue and in higher levels in a number of malignancies. In previous studies, Glut-1 overexpression has been found in breast cancer and has been associated with higher grade and proliferative activity. The aim of our study was to characterize the immunohistochemical (IHC) expression of Glut-1 in the morphologic and molecular subtypes of invasive breast cancers.

Design: We identified 523 cases of invasive breast cancer from the pathology database between 2004 and 2006. The clinicopathologic findings and the biologic markers including ER, PR and Her-2 status were reviewed. IHC for CK5/6, CK 14, and Glut-1 were performed on tissue microarray using standard procedures. Positivity for Glut-1 was defined as cytoplasmic staining of more than 40% of tumor cells. Follow up for all the patients was obtained from our records (CIS) and SEER database. Statistical analysis using Chi square, Fisher's exact test, Kaplan Meier was done.

Results: We were able to morphologically classify 415 tumors as basal-like; 54 (10%), and non-basal-like cancers; 361 (90%). Glut-1 was overexpressed in 33 (67%) of basal-like tumors, whereas only 55 (24%) of the non-basal cancers showed increased expression for Glut-1 *(p <0.0001)*.

The correlation between Glut-1 and basal-like breast cancer

Glut-1	Basal-like	Non-basal
No.of cases	49	231
Positive	33(67%)	55(23%)
Negative	16(33%)	187(73%)
p value	< 0.0001	

Glut 1 overexpression correlated also strongly and significantly with tumor grade within the two categories. Among the poorly differentiated tumors, Glut-1 was significantly overexpressed in 29 (71%) of the basal –like cases, in contrast to the non-basal type which showed increased expression in 32(29%) cases only *(p<0.0001)*. Furthermore, Glut-1 overexpression was associated overall with increase tumor size *(p=0.04)*, however, there was no correlation between the overexpression of Glut-1 and patients outcome for the two subtypes.

Conclusions: We determined the overexpression of Glut-1 in basal-like in comparison to non-basal-like cancer. Glut-1 may serve as a potential prognostic marker and a therapeutic target in this aggressive subgroup of breast cancer.

224 Does Her2neu Expression Vary with Fixation Time?

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Background: It is said that Her2neu expression by IHC varies with the time of fixation in neutral buffered formalin. However, there are no published data validating this claim. The published guidelines[1] from ASCO and CAP recommend that the tissue fixation window should be between 6 and 48 hrs in order to reliably perform IHC for Her2neu. In many pathology practices this guideline can present logistic problems, particularly with cases received on Thursday and Friday.

Design: The goal of this pilot study is to examine 10 cases. Punch biopsy samples (3 mm) from tumors removed as part of patient's treatment were obtained immediately after receipt of the lumpectomy or mastectomy in the laboratory. These samples were immediately placed in 10% buffered formalin for 3 hrs, 48 hrs, 72 hrs, 96 hrs and 120 hrs. The tumors were large enough that the small samples removed did not compromise the analysis of the case. The study samples were not stained until the case was completed. After the fixation periods, each block was immediately processed. All cases, except for 5,7 and 10 were processed for 2:45 hrs in a Shandon Excelsior tissue processor. Case 5-48 hrs and case 10-3hrs were processed for 4 hrs in a Leica Peloris tissue processor. The remainder of cases 5, 7, and 10 were processed overnight for 11 hrs. All blocks were then batch stained with PATHWAY Her2neu Clone 4B5 rabbit monoclonal using Ventana Ultraview DAB detection kit in a Ventana Benchmark™ XT processor. All cases were invasive carcinomas known to be Her2neu overexpressers before inclusion in the study. Two pathologists reviewed every slide independently following the ASCO/ CAP guidelines [1].

Results: Eight cases of invasive breast carcinoma have been analyzed thus far. All blocks, regardless of whether they were fixed for 3, 48, 72, 96, or 120 hrs had strong and diffuse membranous staining with Her2neu. No significant staining difference was noted between the various fixation times.

Conclusions: Fixation times in 10% buffered formalin between 3 and 120 hrs do not affect Her2neu expression. Further studies are needed to confirm this finding. **Reference:** American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med 2007;131:18-43.

225 Combined Proteomic-Transcriptomic Profiling of Laser Capture Microdissected Normal and Breast Cancer Epithelium Reveals Systematic Biochemical Network Alterations

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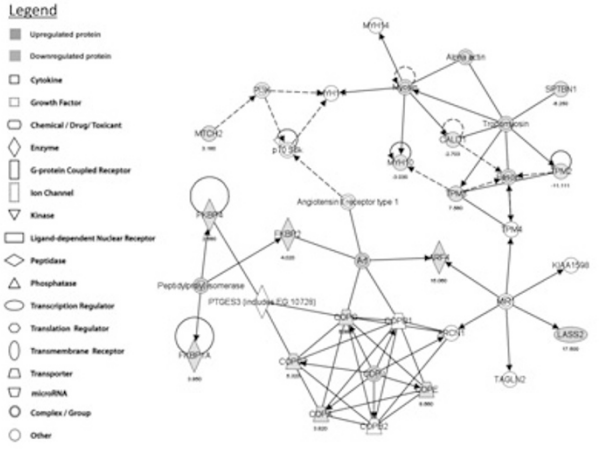
Background: Global profiling studies in solid tumors are ideally carried out on purified cell populations, such as those obtained through laser capture microdissection (LCM). Proteomic analysis of LCM samples has been traditionally limited by the sensitivity of standard analytic methods and the small amounts of tissue isolated through LCM. We have recently optimized an analytic method employing nano-LC-MS/MS to globally profile proteins in LCM tissue with a high degree of sensitivity and quantitative resolution.

Design: We profiled the proteomes of 10 estrogen receptor (ER) positive invasive ductal breast carcinoma (IDC) and 10 mammoplasty LCM specimens. We analyzed significant proteomic alterations in the context of known biochemical networks obtained from the Ingenuity Pathway database and global gene expression profiles measured in a parallel LCM breast cancer dataset.

Results: We identified 468 proteins significantly altered (289 up and 179 down) between IDC and normal epithelium (FDR<0.1). These proteins were related via multiple canonical pathways and biochemical networks obtained from the Ingenuity pathway database. IDC proteomes were altered in cavolear mediated endocytosis signaling ($P<10^{-10}$) and small molecule metabolism (P<0.05) pathways. An ensemble of the most significant ($P<10^{-30}$) protein-derived Ingenuity networks was significantly enriched for genes exhibiting differential mRNA expression between LCM IDC and normal breast epithelium (P<0.05). The network with the highest concordance between protein and gene expression profiles was centered at the angiotensin-II receptor, a druggable target recently implicated in breast carcinogenesis (Figure 1). **Figure 1** Combined proteomic-transcriptomic analysis identifies an altered network around the angiotensin II receptor.



Conclusions: Combined proteomic and transcriptomic profiling of LCM dissected tissue revealed molecular alterations associated with breast cancer. These alterations cluster into networks that suggest novel pathophysiology and diagnostic / therapeutic targets.

226 Concordance between Semi-Quantitative Immunohistochemical Evaluation and Oncotype Dx RT-PCR Derived ER, PR and HER-2 Status in Breast Cancer

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Background: Oncotype Dx is a commercially available 21-gene set expression profile by reverse transcriptase polymerase chain reaction (RT-PCR) in Stage I & II, estrogen receptor (ER) positive breast cancer (BC). In addition to reporting the recurrence score as high, intermediate and low risk, Oncotype Dx report also includes ER, Progesterone receptor (PR) and Her-2 neu scores derived by RT-PCR. In this study, we aimed to correlate Oncotype Dx RT-PCR based ER, PR and HER-2 with immunohistochemistry (IHC) derived ER, PR, and HER-2 scores.

Design: We reviewed the pathology reports on BC cases submitted for Oncotype Dx and recorded the ER and PR semi-quantitative immunohistochemistry (IHC) score using the SP1 and SP2 clones respectively. The HER-2 status, determined by IHC (A085 clone) and read by Chromavision automated cellular Imaging system (ACIS), was also retrieved. ER and PR data by both methods was available on 111 cases and HER-2 on 60 cases. Positive ER and PR staining on IHC was divided into 4 semi-quantitative grades, 1 (low positive), 2-10%, 2, 11-25%, 3, 26-75% and 4, >75% tumor cell nuclei immunoreactive. The HER-2 staining score by ACIS was divided in three groups, negative (0 to 1.5), indeterminate (1.5-2.9) and positive for HER-2 (≥3.0). All indeterminate cases on IHC were tested by Fluorescent in-situ hybridization (FISH) for HER-2 gene amplification. In Oncotype Dx, ER >6.5, PR>5.5 and HER-2>11.5 by RT-PCR is considered positive.

Results: ER grade by IHC was 1 in 5, 3 in 5 and 4 in 101 cases. In the PR positive group 22, 9, 21 and 59 were grade 1, 2, 3 and 4 respectively.For HER-2, 41/60 were negative by IHC and 19 were indeterminate (score 1.6 – 2.7), all the indeterminate cases were negative on FISH. There was 97% concordance between ER by IHC and RT-PCR; of the 3 cases negative for ER by RT-PCR, 2 had IHC grade of 1. PR status by RT-PCR showed less concordance with IHC; 23/111 (21%) cases converted from PR positive on IHC to PR negative by RT-PCR. Of these 23 cases PR grade by IHC was 1 in 15. Of the HER-2 negative, only 1/60 was called positive by RT-PCR. In this case FISH was also negative in the invasive component but it had a FISH amplified DCIS in close proximity to the invasive cancer.

Conclusions: Semi-quantitative ER and HER-2 grade by IHC correlates well with RT-PCR score but IHC may be a better test because of morphologic correlations. However, the threshold for PR positivity by IHC and RT-PCR needs to be re-evaluated especially the low PR positivity by IHC.

227 Should Microscopic Incidental Intraductal Papillomas of the Breast Diagnosed on Core Needle Biopsy Be Excised?

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Background: Most authors recommend excision of intraductal papillomas (IDP) diagnosed on core needle biopsy (CNB). This leads to the question of whether excision is necessary for incidental intraductal papillomas (iIDP) on CNB.

Design: Using the pathology computerized data base we retrospectively identified 46 iIDP diagnosed on CNB from 1/2000 to 12/2008. Clinical, radiologic and pathologic information was gathered and correlated. All CNB were reviewed to confirm the diagnosis of iIDP, and excision specimens reviewed when available.

Results: Of the 46 patients, follow up information was available in only 38. The age of these patients ranged from 39 to 82 (mean= 48years). Most iIDP were diagnosed by mammotome CNB(36 cases). 33 cases were performed for calcifications (ca++) with the following indications: clustered=21, new=4, pleomorphic=3, increasing=3, indeterminant=2,. The correlating diagnoses included: fibrocystic changes (FCC) with calcium phosphate=18, calcium oxalate=10, fibroadenoma with ca++=5. The 3 masses were: 2 cases of cystic papillary apocrine metaplasia and 1 fibroadenoma. 1 case was diagnosed via ultrasound and was a fibroadenoma. The last case was diagnosed via MRI and was cystic papillary apocrine metaplasia. In all cases the IDPs were <=0.2cm, were not associated with ca++, and were incidental to them or the underlying mass. 14 patients underwent excision, whereas the remaining 24 have remained radiologically stable for over 12 months. The excision specimen findings were: FCC=8 and IDP=6. With the exception of 1 case, all the IDP persisted to be incidental. In this solitary case, the ca++ were described as pleomorphic and corresponded to FCC ca++ on CNB. However on excision, residual pleomorphic ca++ on mammogram correlated with ca++ in both FCC and IDP. No cases were upstaged on excision to atypical duct hyperplasia or intraductal or invasive carcinoma.

Conclusions: With the exception of 1 case, all iDP diagnosed on CNB were either completely excised or remained incidental. The exception occurred due to sampling error and accounted for the change from an iIDP on CNB to one that was associated with ca++ on excision. Given the complete lack of upstaging, our recommendation is not to excise iIDP diagnosed on CNB provided the index lesion has been adequately sampled and radiologic follow up is maintained.

228 Non-Atypical Papillary Lesions on Core Biopsy: Do All of Them Require Surgical Excision? A Meta-Analysis of 604 Cases

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Background: All papillary breast lesions with atypia on core biopsy are excised because of a high risk of associated malignancy. However there is still controversy regarding the need for excision of all non-atypical lesions. A meta-analysis was performed to estimate the percentage of cases upgraded to DCIS or invasive carcinoma (IC) after immediate surgical excision, and to evaluate possible radiological, and clinical features that may be predictive of malignancy.

Design: Relevant studies were identified using Pub med database. Studies included were those that provided sufficient data to calculate the percentage of cases upgraded, and that excluded the association of other high grade lesions on the biopsy. Parameters recorded included: size and appearance of the lesion on imaging studies, location (central vs. peripheral), needle gauge, number of cores, residual lesion after core Bx (completely excised or not), and whether the papillary lesion was an incidental finding or not.

Results: Twenty three articles were found including 604 cases. Fifty five lesions were upgraded to DCIS or invasive carcinoma (9.1%) in the surgical specimen (30 DCIS, 11 IC, 8 papillary carcinoma, 6 not specified).Eleven studies correlated false negative cases with clinical and radiological features. Two features that some studies reported more commonly in these cases include microcalcifications (MC), and a size ≥ 2 cm. However these features were only evaluated in few studies (MC in 17, size in 5). Needle gauge, complete excision of the lesion by core biopsy, location (central vs. peripheral), number of cores, and whether the papillary lesion was the target lesion or an incidental finding were not evaluated in the majority of studies, although they could be potentially useful in predicting malignancy.

Conclusions: This meta-analysis shows a 9.1% false negative rate for papillary lesions without atypia . The presence of MC within the lesion and a size ≥ 2 cm correlate with an increased risk of malignancy in some studies. However most studies fail to analyze these and other parameters that may aid in predicting malignancy. We recommend including them in any future studies.

229 Atypical Ductal Hyperplasia at 25 Years – Interobserver and Intraobserver Variability

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Background: Atypical ductal hyperplasia (ADH) was formulated as a diagnostic category in 1985 and is lies in between usual ductal hyperplasia (UDH) and ductal carcinoma in situ (DCIS). These categories are associated with varying levels of risk for development of invasive breast cancer. Both morphological and size criteria have been used to differentiate these entities. Rosai et al have demonstrated poor inter-observer reproducibility, while Schnitt et al have demonstrated marked improvement if standard criteria were used. The aims of the study were to investigate the inter-observer and intra-observer variations, and evaluate the improvement in reproducibility by using high molecular weight keratin (HMWK) expression analysis.

Design: Nine pathologists reviewed 81 cases of breast proliferative lesions in a three stage process. The lesion was marked and the pathologists were instructed to only evaluate the marked area as per their usual diagnostic criteria with the exception of the size criterion and assign diagnoses of UDH, ADH or DCIS to each lesion. In the second stage, the H&E slides were re-labeled and re-evaluated, while in the third stage an immunostain for HMWK was also provided. Concordance was evaluated at each stage of the study.

Results: The overall reproducibility among the 9 pathologists for diagnosing the 81 proliferative breast lesions was fair (Kappa value =0.342), whereas when each of the 9 pathologists was compared to each of the other 8 pathologists, the kappa value ranged from 0.152 to 0.568 (slight to moderate agreement). The intra-observer kappa value ranged from 0.561 to 0.883 (moderate to strong). Complete agreement among 9 pathologists was achieved in only 9 (11.1%) cases, atleast 8 agreed in 20 (24.7%) cases and 7 or more agreed in 38 (47.0%) cases. Out of 81 cases, maximum agreement among pathologists was observed in 34 lesions of UDH, 29 lesions of ADH and 13 lesions of DCIS. Equivocal agreement was obtained for five lesions. Following IHC stain a significant improvement in the inter-observer concordance was observed.

Conclusions: ADH still remains a diagnostic dilemma with wide variation in both inter- and intra-observer reproducibility among pathologists. The addition of IHC stains improves the concordance for the diagnosis of these difficult lesions.

230 Epithelial-Mesenchymal Transition in Breast Carcinomas

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Background: Epithelial-mesenchymal transition (EMT) is characterized in loss of epithelial nature and acquisition of mesenchymal nature. EMT is proposed as important step in tumor invasion and metastasis. However EMT may be transient and cannot be identifiable in human tumor. Recent studies suggest that EMT occurs in specific genetic context, basal phenotype of breast carcinomas.

Design: To assess a role of EMT in human mammary carcinogenesis, we performed immunohistochemical studies for EMT markers(vimentin, SMA, E-cadherin, N-cadherin, CK19 and Cam 5.2)using tissue microarray sections.

Results: Total 492 cases were evaluated in this study and classified into hormonal receptor positive (HR) type, Her-2 type, and triple negative (TN) type using immunohistochemistry and *in situ* hybridization. Among the 102 cases of TN type breast cancer, 24.5%, 13.7%, and 9.8% expressed vimentin, N-cadherin, and smooth muscle actin. However, among the 221 cases of HR type breast cancer, 4.1%, 5.9%, and 0.4% expressed vimentin, N-cadherin, and smooth muscle actin, respectively. Decreased expression of epithelial type marker-E-cadherin, CK19, and CAM 5.2-was observed in 16.7%, 45.1%, and 60.8% of TN type breast cancer. Meanwhile, 11.7%, 6.8%, and

3.2% of HR type breast cancer showed decreased expression of E-cadherin, CK19 and CAM5.2, respectively. Loss of epithelial nature and acquisition of mesenchymal characteristic was related to the TN type of breast cancer (p=0.000). It also showed significant association with high histological grade (p=0.000). However, there was no difference in disease free survival of patients whether the EMT was observed or not.
Conclusions: We showed that the EMT is related to the molecular expression profile of breast cancer, particularly, TN type, and high histological grade.

231 Histologic Characteristics of Benign Breast Lesions Identified for Biopsy by Magnetic Resonance Imaging

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Background: Contrast-enhanced magnetic resonance imaging (MRI) of the breast is being used more frequently as a screening modality for high-risk patients. Although quite sensitive, it suffers from poor specificity. In this study we sought to evaluate histologic features of breast biopsies diagnosed as benign that may correlate with the abnormal signal enhancement seen on MRI studies.
Design: The cases records of the Beth Israel Deaconess Medical Center were searched for biopsies of benign breast lesions identified as displaying abnormal or suspicious features (Breast Imaging and Reporting Systems (BIRADS) 4a, 4b or 4c) by MRI. The H&E stained slides were reviewed and evaluated for the following features: diagnostic lesion, prominent vascularity (intra- and extralesional), large-caliber vessels, proportion of fatty to nonfatty tissue, and epithelial versus stromal predominance in nonfatty tissue. Additional clinical features were also examined.
Results: Thirty-seven cases of benign breast lesions categorized as abnormal or suspicious by MRI were identified. Forty-nine percent were categorized as BIRADS 4a, 38% as BIRADS 4b and 13% as BIRADS 4c. Five cases (13%) showed specific diagnostic features of mass-forming lesions, including four fibroadenomas and one papilloma. The remaining cases showed benign breast tissue with or without fibrocystic change. Most cases (92%) displayed stromal-predominant rather than epithelial-predominant nonadipose tissue. Forty-nine percent of biopsies consisted predominantly of fatty tissue. One (3%) of 31 cases with lesional tissue showed prominent intralesional vascularity and two (5%) of the 37 cases showed prominent extralesional vascularity. Twelve (32%) cases showed large caliber vessels, all but one case identified within adipose tissue.
Conclusions: Increased vascularity, the expected histologic correlate for MRI enhancement, was only identified as a prominent feature in a small minority of the studied benign cases. Large-caliber vessels were identified within adipose tissue in 33% of cases; however, it is unclear whether this correlates with specific signal enhancement by MRI. Most cases showed non-specific features of benign breast tissue, predominantly stromal changes, without impressive histologic differences from normal breast tissue. Perhaps technical factors (i.e. fat suppression, sampling) rather than inherent vascular changes may be responsible for signal enhancement in these cases.

232 A Comparison of HER 2 Expression in Primary Breast Carcinomas vs Organs of Metastasis *A Comprehensive Retrospective Review*

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Background: HER2 overexpression is seen in up to 18% of primary breast carcinomas. Data on the conservation of HER2 status specifically in distant metastases is limited. Our aim is to examine the patterns of HER2 staining in distant metastases as compared to the primary tumor and to determine if differences exist between sites of metastases.
Design: Cases were selected from our electronic archives of 2000-09 using 3 criteria: (1) confirmed metastasis (met) from a breast primary, (2) exclusion of loco-regional mets, and (3) HER2 analysis on both the primary and met. Immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) results originally on record were used. Metastatic site groups (bone, serosa-associated sites, solid organ, and non-locoregional lymph nodes) were compared.
Results: 156 cases were identified with HER2 analysis on both the primary and met. IHC was done on 150 (96%) of the primary tumors and 145 (93%) of the mets. FISH was done on 38 (24%) and 47 (30%) of the primary and mets, respectively. In 68 specimens, either primary or met, both IHC and FISH were done. We found a discrepancy between the IHC and FISH in 3 (4%). HER2 was amplified in 52 (33%) and 51 (33%) of the 156 primary tumors and mets, respectively (95% CI 26-42% for both). Ignoring borderline results, 146 cases had HER2 results on both the primary and met. Concordance in HER2 overexpression between primary and met was 82% (95% CI 75-88%) overall, in bone 79%, solid organs 86%, serosa-associated sites 83%, and lymph nodes 64%.

Primary Tumor HER2 (-)

Metastatic Site	HER2 (+)	HER2 (-)
Bone	5	19
Solid Organ	6	41
Serosa	1	15
Lymph Node	1	8

Primary Tumor HER2 (+)

Metastatic Site	HER2 (+)	HER2 (-)
Bone	4	1
Solid Organ	28	5
Serosa	4	3
Lymph Node	1	4

Results for met sites are summarized in **Tables 1**&**2.** There was no statistically significant difference in the HER2 positive proportion between primary and met either for all cases taken together (p=1.00) or for met sites separately (bone p=0.22, solid organ p=0.63, serosa p=1.00, lymph node p=0.37). Additionally, pairwise comparisons between met sites did not reveal statistically significant differences in HER2 overexpression (data not shown).
Conclusions: HER2 overexpression is preserved in distant metastases of breast cancer, which may have implications in its treatment. Additionally, HER2 overexpression does not correlate with metastasis to any particular site.

233 Expression of Transcription Factors [FOXA1, GATA-3] in Estrogen Receptor Negative (ER-) and Progesterone Receptor Positive (PR+) Groups: A Single Hormone Receptor Subset Analysis

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Background: Estrogen receptor (ER) plays a key role in development and influences treatment outcome in breast cancer patients. Ligand binding assays have revealed a small subset of the hormone receptor (HR) category which is single receptor positive. Recently functional interactions between FOXA1 transcription factor of the fork head family and ER are shown to play a critical role in suppressing ER-dependent breast cancer cell growth and tumorigenesis in vivo. This suggests that FOXA1 may be a novel marker that can inhibit and prevent tumor proliferation and development of invasion. Similarly GATA family transcription factors are shown to be highly expressed in the mammary epithelium; exclusively in the luminal epithelial cells. Targeted deletion of GATA-3 leads to profound defects in mammary development and the inability to specify and maintain the luminal cell fate shown in mouse models. In breast cancer, GATA-3 has emerged as a strong predictor of tumor differentiation, ER status, and clinical outcome. The specific aim of this study is to analyze the expression of novel biological transcription markers FOXA1 and GATA-3 in ER-/PR+ breast cancers.
Design: Twenty five (25) cases of the ER-/PR+ group were selected from our archives. Criteria defining the ER-/PR+ group was (1) absent ER expression by two clones and (2) positive for PR expression. Nuclear staining of 10% or more of the tumor cells defined a positive FOXA1 and GATA-3. A cumulative "H score" is derived based on proportionality and intensity scores [H = 0 negative, 1-150 low, 151-250 intermediate, 250-300 high]. All results of ER and PR were confirmed by two clones for both ER {6F11/ SP1} and PR {1A6 and 1E2} on the Benchmark XT according to the FDA protocol. Staining for PR clone 636 was also performed on a DAKO auto stainer and FDA protocol.
Results: Moderate to strong GATA-3 expression was seen in 23 cases (92%; H score mean = 110, range 5-210). On the other hand low FOXA1 expression was seen in 21 (77%; H score mean = 27, range 5-130).
Conclusions: 1. Moderate to strong expression of GATA-3 in seen in up to 92% of ER-/PR+ cases, suggesting the expression of transcription factors in ER- tumors. **2.** Expression of FOXA1/GATA-3 in ER-/PR+ cases may play an important role in the hormone responsiveness of these tumors. **3.** FOXA1 and GATA-3 may offer new promising targets for therapy in this small, hormone unresponsive group.

234 Frozen Section Versus Touch Preparation for Intraoperative Detection of Sentinel Lymph Node Metastases from Breast Cancer: A Single Institution Experience of 396 Cases

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Background: The single most important predictor of outcome for women with breast carcinoma is the status of the ipsilateral lymph nodes. Frozen section (FS) method applies excessive tissue sectioning which might deplete a focus of tumor metastases. The purpose of this study was to compare tumor size in SLN on FS vs. permanent section (PS).
Design: Intraoperative touch preparation (TP) or FS was performed on SLN of consecutive breast cancer patients from 2007 to 2009. The decision TP vs. FS was totally dependent on the pathologist preference. When FS was decided, 2 consecutive sections were prepared. Tumor metastases size on FS and PS were recorded. Sensitivity, specificity and overall accuracy of detecting positive SLN were calculated for FS and TP groups. Fisher's exact test, Wilcoxon rank sum test and Spearman correlation test were used for statistical analyses.
Results: There were 396 patients with SLN biopsy, 124 (31.3 %) patients had at least one positive SLN. While FS was performed on 73 patients, TP was performed on 323 patients. While the specificity was 100% for both procedures, TP had sensitivity of 54.5% and FS had sensitivity of 89.4%. The median and range of the size of the tumor metastases is outlined in Table 1.

Median and range of tumor metastases for each subgroup

	Median (mm)	Range (mm)	P value
FS (TrP)	5	0.25 to 11	<0.0001
FS (FN)	1.5	1 to 5	
TP (TrP)	7.5	0.09 to 27	
TP (FN)	1.5	0.3 to 10	

FS, frozen section; TP, touch preparation; TrP, true positive; FN, false negative

A total of 49 positive SLNs were examined intraoperatively by FS. The tumor size on FS and PS were highly correlated (Spearman correlation coefficient 0.87 (p<0.0001). However, 3 (6.1%) cases were positive on FS and negative on PS. One case became micrometastases on PS after it was macrometastases on FS.
Conclusions: Frozen section is superior to TP as a method of testing SLN. However, 6.1% of positive SLN on FS become negative on PS. This raises the possibility that some negative SLNs on FS might have been underdiagnosed. Therefore, when FS is used, some patients could be falsely down-staged.

235 Histologic and Immunophenotypic Comparison of Estrogen Receptor (ER)-Positive Breast Cancers in *BRCA1* Mutation Carriers and Sporadic ER-Positive Breast Cancers: A Case-Control Study

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Background: Most invasive breast cancers (IBC) in *BRCA1* mutation carriers are ER negative (-). These tumors are most often high grade invasive ductal carcinomas and have a basal-like phenotype. However, up to one-third of *BRCA1*-associated IBC are ER positive (+). We previously reported that ER+ *BRCA1*-associated IBC show a wider spectrum of histologic types and grades than ER- IBC that occur in this setting, raising the possibility that at least some of these ER+ IBC may be sporadic rather than mutation-related. However, how the features of ER+ *BRCA1*-related IBC compare directly to sporadic ER+ IBC has not been previously studied.

Design: We performed a case-control study of 60 ER+ IBC arising in women with *BRCA1* germline mutations (cases) matched on age and year of diagnosis with 174 ER+ sporadic breast cancers (controls). Histologic features were reviewed and tissue microarrays (TMAs) were constructed and immunostained for CK5/6, CK7/8, CK14, CK18, CK19, EGFR, and HER2. Immunostain results were analyzed by unsupervised hierarchical clustering using Cluster and Treeview programs.

Results: ER+ *BRCA1*-associated IBC were significantly more likely than sporadic ER+ IBC to be invasive ductal type (p=0.005), histologic grade 3 (p=0.006) and to have a high mitotic rate (p=0.0003). In addition, ER+ *BRCA1*-associated IBC were 4-times more likely to express basal cytokeratin CK14 than ER+ sporadic IBC (13% versus 3%, respectively, p = 0.02). On unsupervised cluster analysis, ER+ *BRCA1*-associated IBC were heterogeneous with regard to their biomarker expression profile; some clustered more closely with sporadic ER+ IBC whereas others clustered more closely with ER- *BRCA1*-associated IBC.

Conclusions: The results of this study show for the first time that ER+ breast cancers arising in women with *BRCA1* germline mutations show several morphologic and immunophenotypic differences from ER+ sporadic breast cancers. This raises the possibility that some ER+ *BRCA1*-associated invasive breast cancers are mutation-related and others are sporadic or that there is a unique mechanism by which ER+ cancers develop in mutation carriers. Additional immunophenotypic and molecular studies are underway to further characterize this group of tumors.

236 Atypical Lobular Hyperplasia in Surgical Margins and Needle Biopsies: A Clinicopathological Study in One Institute

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Background: Lymph node negative T1 and Tis carcinomas, and atypical lesions in breast specimens, are increasing. Clinical management of detected atypical lesions without associated more aggressive lesions both in needle biopsies and in surgical margin, is still controversial.Some reports maintain that lobular neoplasia are not only a risk factor for invasive carcinoma, but is also a nonobligate precursor.

Design: During the period 2002-2008, 2,976 needle biopsies, 1,335 intraoperative margin evaluations and 1,724 breast surgeries were performed in our institute. We studied cases with atypical lobular hyperplasia (ALH) and ductal involvement of cells of ALH (DIALH) in needle biopsies and at final margins. ALH and lobular carcinoma in situ were differentiated using Page's criteria. E-cadherin immunostaining was used when it was difficult to distinguish between lobular and ductal neoplasia.

Results: Eleven patients with ALH and/or DIALH without associated malignancy were diagnosed by needle biopsies. No pleomorphic ALH was found at the needle biopsy nor at the margins. Four patients did not undergo excisional biopsy and had no suspected imaging changes (follow up 6 months to 4 years). Excisional biopsies were performed on 7 patients. The diagnoses were: Invasive ductal carcinoma(1), ductal carcinoma in situ(2), atypical ductal hyperplasia(1), ALH(2) and benign lesion(1). ALH and/or DIALH extended to the margins in 46 of 287 cases containing ALH in surgical specimen. Therapies were: Radiation (29), hormone therapy (31) and/or chemotherapy(14). Four cases have been followed up without developing imaging lesions for 8 to 78 months (median: 41 months). Two patients in their 40s had recurrences after radiation therapy. One showed multiple carcinomas and the other recurred in the muscle. Neither recurrence was at the surgical site.

Conclusions: We recommend excisional biopsy following a diagnosis of ALH by needle biopsies in order to rule out malignancy. However, when ALH is present at the margins of a surgical and/or intraoperation specimen, intensive follow up using imaging is possible without the necessity of additional surgery.

237 Neuroendocrine Ductal Carcinoma *In Situ* (NE-DCIS) of the Breast – A Distinct Variant of DCIS

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Background: The World Health Organization (WHO) classifies neuroendocrine tumors (NETs) of the breast as a special tumor entity. However, neuroendocrine ductal carcinoma *in situ* (NE-DCIS) of the breast, which could be considered a precursor lesion of breast NETs, has not been adequately studied and its clinicopathological significance remains poorly understood. Therefore, we investigated the clinical and pathological characteristics of NE-DCIS in comparison with those of non-NE-DCIS.

Design: NE-DCIS was diagnosed according to the WHO classification criteria for breast NETs, i.e. DCIS tumors in which more than 50% of the cell population immunohistochemically expressed NE markers (chromogranin A and/or synaptophysin) were diagnosed as NE-DCIS. We clinicopathologically analyzed 20 NE-DCIS and 274 non-NE-DCIS cases.

Results: NE-DCIS accounted for 6.8% of all DCIS. The mean patient age was 50.4 years for NE-DCIS and 49.6 years for non-NE-DCIS (p=0.66). The main clinical presentation of NE-DCIS patients was bloody nipple discharge, seen in 72% (13/18) of cases, significantly different from the 5% in non-NE-DCIS patients (p<0.01). Neither metastasis nor recurrence was seen in the NE-DCIS cases (mean 67 months post surgery). Carcinoma was preoperatively diagnosed in 67% (12/18) of NE-DCIS, significantly less than the 95% in non-NE-DCIS (p<0.01). Characteristically, the histological architecture of NE-DCIS was a predominantly solid growth of cancer cells, frequently with well-developed vascular networks. Cancer cells in NE-DCIS had fine-granular cytoplasm and ovoid, occasionally spindle-shaped, nuclei. The nuclear grades and Van Nuys classification were significantly lower for NE-DCIS than for non-NE-DCIS (p<0.01). Immunohistochemically, the mean MIB-1 labeling index of NE-DCIS was 4.3%, significantly lower than the 8.1% in non-NE-DCIS (p<0.01). Furthermore, NE-DCIS scored significantly higher for estrogen and progesterone receptors in the Allred scoring system and lower for HER2, compared with non-NE-DCIS (p<0.01).

Conclusions: NE-DCIS of the breast has characteristic clinical and histological features and could therefore be regarded as a distinctive entity, a NE variant of DCIS. We advocate careful consideration of diagnosis and treatment for this presumably indolent breast cancer. NE-DCIS is the significant disease entity in clarifying and understanding a natural history of breast NETs.

238 Ki-67 Proliferative Index in Estrogen Receptor-Positive/HER2-Neu Negative Early Breast Cancer Predicts Oncotype DX Recurrence Score

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Background: Oncotype DX assay is a useful tool to calculate the risk of recurrence in early breast cancer patients, with prognostic and therapeutic implications. However, the cost of the assay may not be affordable by many patients, leading us to search for more cost effective method. We examined relation between the histopathologic variables, hormonal receptors IHC score, Ki-67 proliferation index as predictors for Oncotype DX recurrence score in estrogen receptor (ER)-positive, Her2/neu negative early breast cancer patients.

Design: Slides and surgical pathology reports from 84 cases of breast carcinomas evaluated by Oncotype DX were retrospectively reviewed to determine patient age, tumor size and histologic grade. All cases were LN negative, ER positive and Her2/neu negative. Cases were immunostained for Ki67 proliferative marker. Estrogen and progesterone receptors (ER and PR) and Ki67 were reported as a semi-quantitative score reflecting the proportion of positive cells.

Results: Eighty four patients were included in the study, age range 38-74 with a mean 51$\pm$8 yrs. Onctotype DX recurrence score (RS) were divided into low risk 42/84 (50%) cases, intermediate risk 27/84 (32%) and high risk in 15/84 (18%) cases. The value of Ki67 index was follows: 16$\pm$10 in Low risk group, 37$\pm$12 in intermediate and 60$\pm$11 in high risk group with significant difference (P< 0.001). On histologic examination, 17/84 (20%) showed low histologic grade, 56/84 (67%) intermediate grade and 11/84 (13%) cases high histologic grade. Lymphovascular invasion was identified in 18/84 (21%) cases. PR was negative in 8/84 (10%). There was significant correlation between RS and Ki67 (r=0.75, P< 0.0001), high histologic grade (r=0.41, P< 0.001), and negative staining with PR (r=0.48, P< 0.001).

Conclusions: The present study shows the importance of cell proliferation as a determinant of biologic behavior of breast cancer in ER positive/Her2neu negative early breast cancer. Measurement of ki-67, histologic grade, and PR negativity are correlated with RS and may be helpful for physicians to detect high-risk patients and to adopt appropriate procedure such as adjuvant therapy.

239 Gene Expression Signature as a Predictor for Trastuzumab Resistance in Breast Carcinoma with Amplified *HER2*

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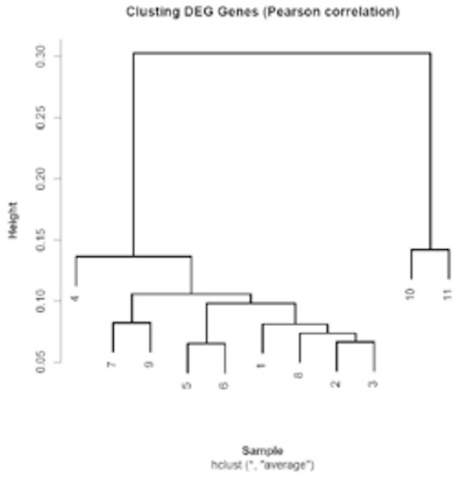
Background: Recent trials have demonstrated remarkable efficacy from combined Trastuzumab and chemotherapy in the adjuvant setting of breast cancer. However, Trastuzumab resistance continues to be problematic. We intended to explore the possibility of presence of distinctive gene signature for Trastuzumab responders versus non-responders.

Design: Thirty two consecutive breast carcinoma cases that had amplified *HER2* were collected. Clinicopathologic data including patients' age, race, tumor type, grade, size, hormon receptor status and node status as well as therapy modality and disease free and overall survival were recorded. Trastuzumab response was defined as no tumor recurrence for more than 3 years after therapy. RNA was extracted and profiled using Illumina Human HT-12 v3.0 whole genome gene expression array. The microarray data were analyzed to identify differentially expressed genes (DEGs) –based and pathways-based gene signature of Trastuzumab response.

Results: There were 21 patients treated with Trastuzumab; 9 responders, 2 non-responders and 12 patients had time less than 3 years follow-up after therapy. There were 15 patients ER positive and 17 patients ER negative cases; 16 patients >50 year-old; 7 patients black, 23 patients white and 2 patients with unknown race. There were 15 patients with early stage and 17 with advanced stage. For the Trastuzumab treatment responders vs. non-responders comparison, there were a large number of DEGs (~900) with significant *P-value*. Of those, there were 125 genes with at least 2 fold up-regulation and 44 genes with at least 2 fold down-regulation. Both principal component analysis and hierarchical clustering suggest that these DEGs can separate these patients into two distinct groups consistent with their Trastuzumab treatment phenotypes (*i.e.*, responder vs. non-responder) [figure]. HER2 signaling pathway was significantly dis-regulated when Trastuzumab responders vs. non-responders were compared (*P*< 0.025, Kolmogorov–Smirnov test). These genes were GRB2, PLCG2, RPS6KB1 and CBLB.

Conclusions: Our genome-wide expression profiling suggests that Trastuzumab responders have distinctive gene signature comparing with non-responders.



240 HER2 Testing: How To Get High Concordance Rate between Immunohistochemistry and Fluorescence In Situ Hybridization

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Background: ASCO-CAP 2007 has expanded the equivocal HER2 by immunohistochemistry (IHC) to include cases that have intense complete staining in <30% of tumor cells instead of <10%. The purpose of this study was to find how a high concordance rate between IHC and fluorescence in situ hybridization (FISH) can be achieved.

Design: A series of 452 breast cancer cases were retrieved from our files from 2007 to 2009. All cases were tested for HER2 (DAKO rabbit polyclonal antibody A805). 2+ category was expanded to include cases that had intense complete circumferential staining in <90% instead of ASCO-CAP's criteria of <30% and incomplete weak or non-uniform staining in >10% which would have been designated as 1+ by ASCO-CAP. These cases were tested by FISH (pathVysion, Vysis). FISH results were scored as not amplified, equivocal or amplified when the HER2/Cep17 ratio was < 1.8, 1.8-2.2, or > 2.2, respectively. In addition, HER2 FISH was performed on 40 cases of 3+ and 61 cases of 0 or 1+ that were randomly selected.

Results: Using our criteria, there were 51 (11.3%) 3+ cases, 185 (40.9%) 2+ cases, 72 (15.9%) 1+ cases and 144 (31.9%) 0+ cases. Using ASCO-CAP criteria, there were 72 (15.9%) 3+ cases, 133 (29.4%) 2+ cases, 103 (22.8%) 1+ cases and 144 (31.9%) 0+ cases. Therefore, there were 52 (11.5% of total) additional cases that were reclassified as 2+ (31 from 1+ and 21 cases from 3+). FISH results on the additional 52 cases are illustrated in [table]. While, this expansion on the lower end of the spectrum (1+ category) identified 1 case with *HER2* amplification, the expansion on the higher end (3+ category) identified 3 cases with borderline *HER2* and 7 cases with non-amplified *HER2*. The concordance rate in the randomly selected cases between IHC and FISH was 100%.

Conclusions: By expanding the 2+ category (11.5% of cases were re-classified as 2+) to include cases that have intense complete staining <90% instead of <30% of cells and 1+ cases that have >10% weak or non-uniform incomplete staining, the concordance rate between IHC and FISH could reach 100%.

FISH results on the additional 2+ cases based on our criteria

	FISH>2.2	FISH 1.8-2.2	FISH<1.8	total
IHC (1+)*	1	0	30	31
IHC (3+)*	11	3	7	21

* 1+ incomplete weak or non-uniform staining in >10%; 3+ complete intense staining in <90%

241 Relation of Hormone Receptor and Her2 Expression to Apocrine Cytology in 305 Grade III Breast Carcinomas

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Background: The incidence and significance of apocrine cytology (AC) in grade III breast carcinomas (BC) is uncertain. cDNA microarray studies have identified BC cases with increased androgen signaling among the Her2 molecular classes. These BC exhibit strong AC features and androgen receptor (AR) expression, suggesting a link between Her2 signaling and the "molecular apocrine" phenotype.

Design: To examine the relationship of AC to Estrogen receptor (ER) and Her2 expression in high grade BC, we identified 305 cases of grade III BC (1993-2007). All cases were evaluated for AC with standard H&E stain. Criteria for AC were 1) large cells with abundant eosinophilic cytoplasm and a N/C ratio of ≥ 1:2; 2) sharply defined cell borders; 3) large vesicular nuclei with prominent nucleoli. Her2 was determined by 3+ immunohistochemical staining (IHC) or fluorescence in-situ hybridization. ER expression was determined by IHC. Cases with > 25 % AC were tested for androgen receptor (AR) by IHC. The tumors were immunophenotyped into molecular subtypes: Luminal A (ER and/or PR+, Her2-), Luminal B (ER and/or PR+, Her2+), Her2 (ER/PR-, Her2+) or Basaloid (triple negative).

Results: 109/305 patients (36%) had AC; 83/109 were stained for AR; 26 were AR+ (31%). ER was expressed in 33/109 patients (30%) with AC, and in 120/196 without AC (61%). 25 AR+ patients 7 (28%) expressed ER, and of 3/54 AR- patients (5.6%) expressed ER. Her2 expression was known in 258 patients. 54/94 patients with AC (58%), and 73/164 without AC (45%) expressed Her2; 20/25 AR+ patients (80%) and 22/45 AR-, AC patients (49%) expressed Her2. AC+ and AC- tumors were respectively, 9.5% and 31% luminal A, 16% and 27% luminal B, 42% and 17% Her2, and 33% and 24% basaloid. 15/25 AR+ (60%) were Her2 type; AC was present in 15% luminal A, 25% luminal B, 58% Her2, and 44% basaloid tumors. 32% AC+ and 40% AC- patients' stages were III or IV, but 16/26 AR+ patients (62%) were stage III or IV.

Conclusions: AR+ tumors were distinguished from AR- AC tumors by having ≥75% AC and from tumors without AC by low ER and high Her2 expression. Most AR+ tumors were Her2 type. Grade III BC with predominant AC and AR+ may represent a distinct, aggressive pathological subtype of BC NOS whose response to therapy might be different.

242 Demographics and Tumor Characteristics of Breast Cancer in Young Women: Evidence of Racial Disparity

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Background: Studies have shown that younger women with breast cancer have worse survival than older women, which may be an expression of underlying more aggressive tumor biology. In addition many invasive breast carcinomas in African–American (AA) women belong to the so called "triple negative" subtype that is associated with worse overall prognosis. In this study we evaluated the demographics and tumor characteristics of young women with invasive breast carcinomas to examine the hypothesis that young AA breast cancer patients have more aggressive tumor characteristics than their young white counterparts.

Design: Our population consisted of 140 consecutive patients <40 years of age (mean 34, range 20-39) diagnosed with invasive breast carcinomas from 2003-2009. Electronic medical records were reviewed to determine demographics and pathologic tumor characteristics including lymph node status, histologic grade and type, size, and breast marker profile (ER, PR, HER-2, EGFR and MIB-1).

Results: Six (4%) of 140 patients were 20-24 years old, 18 (13%) were 25-29 years old, 35 (25%) were 30-34 years old and 81 (58%) were 35-39 years old. 76 (54%) were White, 44 (31%) were AA, 11 (8%) were Hispanic, 6 (4%) were Asian and 3 (2%) were of other races. Invasive ductal carcinoma was diagnosed in 120 (86%), lobular in 2 (1%), mixed ductal and lobular in 15 (11%) and 3 (2%) had other tumor types. The average tumor size was 2.3 cm. 84 (60%) of these young patients had grade III tumors and 61 (53%) had positive lymph node status. Only 52% of the patients were ER positive and only 44% were PR positive. 30% were HER-2 positive, 24% were EGFR positive, and 64% had an unfavorable MIB-1 proliferative index. AA patients had a higher number of ER negative (30/44 vs 27/70, p=0.002), grade III tumors (34/44 vs 44/75, p=0.03) than white patients. These tumors tended to be PR negative (29/43 vs 35/70, p=0.06). There was no significant difference in lymph node status, histologic type, average size, HER-2 and EGFR expression.

Conclusions: 1.Young (<40 years old) breast cancer patients have a higher rate of positive lymph node status, and poorly differentiated grade III, ER negative/PR negative tumors than women more than 50 years old (as based on SEER data). 2. Young AA patients have an even higher number of ER negative, grade III tumors than young white patients. The results of this study suggest that the worse biologic characteristics of invasive carcinomas in AA patients are already evident even in the young women cohorts.

243 Mutational Profiling of Phyllodes Tumors

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Background: Phyllodes tumors (PTs) represent 0.3-1% of all breast tumors and have a spectrum of histologic appearances and clinical outcome. PTs are morphologically divided into three categories-benign, borderline, and malignant. Investigators have studied biologic markers as adjuncts to morphology in predicting the behavior of these tumors. For instance, KIT (CD117) overexpression has been noted in malignant PTs in few small studies; however, no *KIT* activating mutations have been identified to date. The aim of this study was to screen for activating mutations in tyrosine kinases and signalling pathway intermediates in PTs. Characterization of specific genetic alterations could help understand the pathogenic mechanisms involved in the initiation and progression of PTs, and ultimately help guide rational therapeutic strategy.

Design: Genomic DNA was extracted from formalin-fixed, paraffin embedded tissue of 26 PTs (10 benign, 9 borderline, and 7 malignant) and screened for a panel of known hotspot mutations using PCR and mass-spectroscopy analysis (Sequenom MassARRAY). The mutation panel covers 321 mutations in 30 genes, including *ABL, AKT1/2/3, BRAF, CDK4, CTNNB1, EGFR1, ERBB2, FBX4, FBXW7, FGFR1/2/3, FLT3, GNAQ, HRAS, JAK2, KIT, KRAS, MAPK2K1/2, MET, NRAS, PDGFRA, PIK3CA, PTPN11, RET, SOS1,* and *TP53.*

Results: Mutation profiling identified the substitution *FBX4 S8R* in 3 of the 26 PTs (one benign and two borderline cases). One of the seven malignant PTs was found to have *MET T992I* substitution. No *KIT* mutations were identified.

Conclusions: Our study identified *FBX4 S8R* and *MET T992I* substitutions that likely represent single nucleotide polymorphisms in a limited number of PTs. Nevertheless, a recent study has shown that the *S8R* alteration in the E3 ubiquitin ligase *FBX4* results in diminished cyclin D1 degradation, and hence increased cyclin D1 protein expression.

The *T992I* substitution in the hepatocyte growth receptor (*MET*) is of unknown functional importance. In conclusion, PTs lack oncogenic mutations that are commonly observed in other types of sarcoma, carcinoma, melanoma, and hematopoietic malignancies.

244 Expression of Estrogen Receptor β Isoforms in Male and Female Breast Carcinoma

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Background: Recent studies have suggested that differential expression of estrogen receptor (ER)β isoforms may have prognostic and therapeutic implications in breast carcinoma. ERβ expression has also been shown in "triple-negative" (ER-, progesterone receptor {PR}-, HER2-) breast carcinoma. Previous studies have focused predominantly on female breast carcinoma (FBC), and data comparing expression of ERβ isoforms in FBC and male breast carcinoma (MBC) are limited. In this study, we compared expression of ERα, ERβ2, ERβ5, PR, and HER2 in FBC and MBC.

Design: Immunohistochemical stains for ERα, ERβ2, ERβ5, PR, and HER2 were performed on tissue microarrays representing 32 MBC (four 1.0 mm cores per case) and 82 FBC (two 1.0 mm cores per case). Nuclear staining for hormone receptors was scored using the Allred system. Membranous HER2 staining was scored according to CAP guidelines.

Results:

Marker	MBC	FBC	p-value
ERα	23/27 (85%)	49/73 (67%)	0.085
ERβ2	2/25 (8%)	3/67 (4%)	0.610
ERβ5	18/23 (78%)	22/67 (33%)	0.0002
PR	14/27 (52%)	40/76 (53%)	1
HER2	6/26 (23%)	14/63 (22%)	1

All ERβ2-positive cases were positive for ERα. Although the majority of ERβ5-positive cases were ERα-positive, ERβ5 was expressed in two of two (100%) triple-negative MBC and in six of 12 (50%) triple-negative FBC.

Conclusions: In our series, ERβ5 expression was significantly more common in MBC than in FBC. This occurred in both ERα-positive and triple-negative carcinomas, although the number of the latter was small. Expression of all other studied markers was similar in both groups. As more is learned about the significance of ERβ subtypes in the pathogenesis of breast carcinoma, the observed differences in ERβ5 expression in MBC and FBC may provide insight about biological differences in MBC and FBC, and provide a potential therapeutic target.

245 Significance of Unamplified Chromosome 17 Monosomy in Breast Carcinoma

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Background: HER2 gene amplification is a poor prognostic indicator in breast carcinoma. Some patients show chromosome 17 aneusomy with or without HER2 amplification. We have in an earlier study demonstrated the poor prognostic effect of unamplified polysomy 17 in breast carcinoma (Mod Pathol 22:1044; 2009). The aim of this study was to determine the effect of unamplified 17 monosomy on prognostic factors in invasive breast carcinoma.

Design: Monosomy 17 was defined as the presence of less than 1.5 chromosome 17 centromere copies/cell. Four groups: **N** (no polysomy or HER2 amplification, 36 cases), M (unamplified 17 monosomy, 23 cases), **P** (unamplified 17 polysomy, 44 cases) and **A** (HER2 amplification without 17 polysomy, 35 cases) were compared for the following: Nottingham score, nuclear grade, mitotic score, histologic grade, lymphvascular invasion, nodal metastases, T stage, estrogen receptor (ER) and progesterone receptor (PR) negativity. SAS 9.2 was used for statistical analysis.

Results: The percentage of prognostic factors is the different groups is given in the table.

% of Prognostic factors

Prognostic factors	% N	% M	% P	% A	N vs. M	N vs. P	N vs. A
Nottingham score 8	11	27	37	34	-	-	-
Nottingham score 9	11	11	14	34	-	-	p=0.05
Nuclear grade 3	26	44	61	74	p=0.001	-	p=0.012
Mitotic score 2	18	33	54	43	-	-	-
Mitotic score 3	18	11	26	40	-	-	p=0.04
Histoogic grade 3	20	37	44	69	-	-	p=0.03
T stage 2	18	11	31	30	-	p=0.03	p=0.03
Lymph vascular invasion present	17	38	19	34	-	-	-
Positive lymph nodes	38	40	42	45	-	-	-
ER negativity	11	17	30	50	-	-	p=0.02
PR negativity	24	48	35	34	p=0.02	-	-

- = not statistically significant

Conclusions: Invasive breast carcinoma with chromosome 17 monosomy is associated with a higher percentage of adverse prognostic indicators such as a higher Nottingham score, nuclear grade, mitotic activity, histologic grade, and lymphvascular invasion compared to cases with neither amplification or 17 aneusomy. However these differences are statistically significant only for nuclear grade 3 and progesterone receptor negativity. The incidence of adverse prognostic factors with unamplified 17 monosomy is less than that seen with unamplified 17 polysomy and even less than that seen with HER2 amplification. The percentage of cases with adverse prognostic factors is : A> P > M > N.

246 Expression, Regulation and Function of Myoglobin in Breast Cancer

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Background: Myoglobin is assumed to be among the best characterized proteins in humans and is widely believed to be restricted to muscle tissues. Non-muscle myoglobin expression has hardly been noticed by a wider public. Having accidentally observed myoglobin expression in solid tumors, we aimed to analyse expression, regulation and function of myoglobin (Mb) in breast cancer.

Design: A wide spectrum of morphological and biological techniques has been used in this study including immunohistochemistry/immunofluorescence, microdissection, transmission electron microscopy, Western blot, qPCR, cell culture (under normoxia and hypoxia), shRNA and siRNA knockdown, *in silico* data mining, high resolution respirometry, migration assays.

Results: Mb protein is expressed in 71% of invasive breast carcinomas (n=917) in association with the hypoxia inducible factor 2a (HIF-2 a), carbonic anhydrase IX and a significantly better prognosis. This expression of Mb in breast cancer was confirmed on mRNA level and occurred irrespective of rhabdomyoid differentiation and was inducible by prolonged hypoxia in breast cancer cell lines (MDA-MB231, MDA-MB468, MCF7) with a median change fold of 3.4 after 72 hours. This induction, mediated by HIF-1- and HIF-2-, originated not from the gene's constitutive promoter active in striated muscle but from an alternative start site proximal to a 5' utr exon that is located upstream the initial ATG codon of the commonly translated reading frame. Functionally, stable myoglobin knockdown in MDA-MB468 cells was associated with a stimulated O_2 uptake during mild hypoxia, yet did not impact the O_2 diffusion gradient in these small cells during limiting conditions. Knockdown of Mb also conferred a significant retardation of the cells' in vitro motility to close an inflicted wound within the normoxic culture.

Conclusions: Mb is commonly expressed in breast cancer at lower levels and irrespective of a metaplastic phenotype. Although generally inducible by hypoxia other functions of myoglobin, not directly related to the transport of oxygen, need to be analysed in further studies.

247 Vascular Lesions of the Breast

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Background: Vascular lesions (VL) of the breast are relatively rare. They may present diagnostic challenge particularly with increased utilization of core biopsy.

Design: We conducted a retrospective study on all VL of the breast diagnosed from years 1992 to 2008. 115 benign VL and 2 angiosarcomas (AS) were identified. Clinical and radiologic data of selected cases were analyzed. Vascular lesions limited to the dermis were excluded from the study.

Results: VL represented 0.34% of all surgical breast specimens (117/34214). There were 73 angiolipomas (AL), 40 hemangiomas, 2 AS, and 2 angioectasias among them. AL in this context is considered to be a vascular proliferation in the fat with the degree of vascularity being variable. Most AL were superficial whereas two cases presented at mid depth. None of the AL were intermixed with benign breast parenchyma in part reflecting superficial location. Diagnosis of lipoma in the breast is difficult to establish but identification of AL on biopsy may avoid unnecessary surgery. Vascular variant of AL poses diagnostic challenge with other vascular tumors with higher vessel density. Other benign VL include cavernous and capillary (lobular and extralobular) hemangiomas. Capillary proliferation in the mammary lobule may be challenging to differentiate from well differentiated angiosarcoma. Two cases of AS followed radiotherapy of breast carcinomas with intervals of 1 and 5 years respectively. Both tumors were of high grade, had multiple recurrences after initial excision and led to the patients' demise.

Conclusions: Benign VL predominate over malignant in the breast. They represent slowly growing mesenchymal neoplasms located at different depths within the breast and some of them include benign breast tissue. Two thirds of benign VL are represented by AL. Cellular AL as well as capillary hemangioma may present diagnostic challenge with differentiation from well differentiated angiosarcoma. Proper identification of AL on needle biopsy may avoid unnecessary surgical intervention, particularly with superficial localization of the mass. Cavernous and capillary hemangiomas are among less frequent breast tumors. Radiographic presentation is dependent on the degree of neoplastic vascularity with higher vascular concentration being visible on mammographic screening. AS of the breast may arise spontaneously or more commonly follow radiation therapy of breast carcinoma, in which case the tumors are mostly of high grade. Normal high vascular and adipose contents of the breast may rarely mimic a benign vascular neoplasm.

248 Molecular Characterisation of Mucinous Carcinomas of the Breast – A Genomic Profiling Analysis

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Background: Mucinous carcinomas have been shown to be transcriptionally distinct from invasive ductal carcinomas of no special type. Here we investigated if mucinous carcinomas would constitute a discrete molecular entity and defined the genomic aberrations that are characteristic of this special type of breast cancer.

Design: 17 mucinous breast carcinomas (12 pure and 5 mixed mucinous and invasive ductal carcinoma of no special type (IDC-NST)) were retrieved from our institutions' histopathology files. All cases were centrally reviewed for assessment of the type, histological grade, mitotic count and presence of a ductal carcinoma in situ. Immunohistochemical analysis was performed on a tissue microarray using a

comprehensive panel of breast markers. Pure mucinous carcinomas were grade- and oestrogen receptor (ER) matched with IDCs-NST (n=24). Representative sections of the tumours were microdissected (in mixed tumours, each component was separately microdissected) and subjected to microarray-based comparative genomic hybridisation (aCGH) using a 32K BAC array platform.

Results: Mucinous carcinomas showed a relatively low level of genetic instability. Unsupervised hierarchical analysis showed that pure mucinous carcinomas were more homogeneous and preferentially clustered together (Fisher's exact test, $p<0.001$). Compared to grade- and ER-matched IDCs-NST, pure mucinous carcinomas significantly more frequently harboured gains in 1p36, 3p21, 4p16, 6p21, 7p22, 8q24, 9q34, 11p15, 11p11, 11q12-q13, 12q24, 16q22, 16q24, 17p13, 17q25, 18p11, 19p, 19q13, 22q13, and loss in 9q11 (Fisher's exact test adjusted p value <0.05). On the other hand, deletions of 16q were significantly less frequent in mucinous cancers. Although 11 pure mucinous carcinomas were of grade I or II, only 3 cases harboured 16q deletion. Finally, the IDC-NST and mucinous components of mixed cases displayed similar patterns of genetic aberrations and clustered together on unsupervised clustering analysis, however the IDC-NST component harboured more genomic alterations than the mucinous component.

Conclusions: Pure mucinous carcinomas are more homogeneous between themselves at the genetic level than IDCs-NST. In mixed mucinous tumours, the IDC-NST component displays a higher number of genomic changes, suggesting that in mixed cases it may evolve from the mucinous component.

249 PPM1D Overexpression and Gene Amplification in Breast Cancers: A QRT-PCR and Chromogenic *In Situ* Hybridisation Study

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Background: *PPM1D* maps to 17q23.2 and encodes a serine threonine phosphatase, which plays a major role in the regulation of the p53 pathway. Our group has recently demonstrated that PPM1D expression and phosphatase activity are required for the survival of cancer cells harbouring 17q23.2 amplification, suggesting that *PPM1D* is one of the drivers of this amplicon. In this study we investigated if *PPM1D* is overexpressed when amplified in breast cancers and the correlations between *PPM1D* overexpression and amplification with clinicopathological features and survival of breast cancer patients.

Design: A cohort of 245 patients with invasive breast cancer treated with therapeutic surgery followed by adjuvant anthracycline-based chemotherapy was retrieved from the files of the authors' institutions. mRNA was extracted from representative sections of tumours containing >50% of tumour cells. TaqMan quantitative real-time PCR using primers for PPM1D and for two house keeping genes was performed. PPM1D overexpression was defined as the top quartile of expression levels. Chromogenic *in situ* hybridisation with in house generated probes for *PPM1D* was performed with observers blinded to PPM1D expression levels and clinicopathological features. Amplification was defined as >5 signals per nucleus or large gene clusters in >50% of cancer cells.

Results: *PPM1D* overexpression and amplification were found in 25% and 6% of cancers, respectively. A strong direct association between PPM1D mRNA expression levels and *PPM1D* amplification was found (Mann-Whitney U test, $p<0.00001$). All cases harbouring *PPM1D* amplification displayed PPM1D overexpression. PPM1D overexpression was associated with oestrogen receptor expression and topoisomerase IIa expression, and inversely correlated with expression of EGFR, and cytokeratins 5/6 and 17. *PPM1D* amplification was significantly associated with HER2 overexpression, and *HER2*, *TOP2A* and *CCND1* amplification. No association between *PPM1D* overexpression and gene amplification with survival was observed.

Conclusions: *PPM1D* is consistently overexpressed when amplified, however PPM1D overexpression is more pervasive than gene amplification. *PPM1D* overexpression and amplification are associated with tumours displaying luminal or HER2 phenotypes. Co-amplification of *PPM1D* and *HER2/ TOP2A* and *CCND1* are not random events and may suggest the presence of a 'firestorm' genetic profile.

250 Dual Color Chromogenic In Situ Hybridisation Seems To Detect a Different Subgroup of HER2/Neu Amplified Immunoscore 2+ Breast Carcinomas Compared to FISH

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Background: Fluorescence in situ hybridization (FISH) is considered the gold standard for the assessment of Her2/neu amplification. In most countries FISH is used to determine amplified breast carcinomas in the group with mild Her2/neu overexpression (immunoscore 2+). The aim of this study was to investigate whether a recently developed chromogenic in situ hybridization (CISH) technique is comparable to a well-established FISH method.

Design: 106 cases of invasive ductal carcinomas of the breast with established Her2/neu status by HercepTest Dako and Vysis FISH were retrospectively analyzed using DAKO DuoCISH. Hercep Test revealed score 2+ for 82 cases, 3+ for 16 cases and 1+ for 6 cases. Formalin fixed, paraffin embedded tissue both from core needle biopsy and from surgical specimens was used (fixation time 16- 48 hours). The Dako DuoCISH uses both a centromere probe for chromosome 17 and a probe for the Her2 gene, which are in a second step coupled with 2 different chromogens. The reaction was carried out on an autostainer according to the manufacturer's instruction. The analysis was performed according to the CAP guidelines by a single investigator using a 100x objective. All discrepant cases were reassessed.

Results: Two score 2+ cases were excluded due to a lack of sufficient tumor tissue. FISH and CISH did not differ significantly with respect to their Her2/CEP17 coefficient ($p=0.9$, paired t test). All score 3+ and 1+ cases were concordant in CISH and FISH (100% amplified and 100% not amplified, respectively). 24 (29%) of the score 2+ cases were amplified by FISH compared to 28 (34%) by CISH, which was not statistically significant ($p=0.45$; chi2 test). Comparing case by case, 12 score 2+ cases (14.6%) were discrepant between FISH and CISH with respect to amplification. 4 cases were FISH amplified/CISH non-amplified, 8 cases CISH amplified/FISH non-amplified. The Her2/CEP17 coefficient of the discrepant cases ranged between 1.2 and 4.75. Of the CISH amplified/FISH non-amplified cases, 7 had a coefficient between 2.09 and 2.75 and 2 showed a heterogeneous amplification.

Conclusions: DAKO DuoCISH seems to be a reliable technique for the assessment of Her2 amplification and allows long-term storage of the slides. The use of light microscopy enables the detection of amplified foci in heterogeneous tumors. However, DAKO DuoCISH seems to detect different Her2-amplified breast carcinomas in the score 2+ subgroup compared to Vysis FISH. Their therapeutic response as well as their outcome will have to be determined.

251 Triple-Negative Phenotype and/or High Mitotic Rate May Improve Selection of High-Risk Patients for BRCA Testing

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Background: Current selection of patients for BRCA testing relies heavily on family history and early onset of breast and ovarian cancer, but improved selection methods are needed because of the high cost of testing.

Design: Two pathologists independently examined multiple histomorphologic features of invasive breast carcinomas in 260 specimens (188 surgicals and 72 core biopsies) from 216 high-risk patients who had undergone BRCA testing. Each feature was scored on a scale of 0 to 2, and associations between individual and combined scores and BRCA mutation status were evaluated.

Results: Morphologic features found by both pathologist to be associated with BRCA1 mutations in 129 untreated surgical specimens were tumor circumscription (Fisher's exact test, $P=0.005$ and $P=0.001$), tumor necrosis ($P=0.026$ and $P=0.007$), and a high mitotic rate ($P<0.001$ for both pathologists). Triple-negative phenotype and tumor grade were also associated with BRCA1 mutations ($P<0.001$ and $P=0.003$, respectively). None of the patients with low-grade tumors had BRCA1 mutations. A total morphology score of ≥4 was associated with a BRCA1 mutation ($P<0.001$ for both pathologists), but a high mitotic rate alone was better able to predict a BRCA1 mutation. A mitotic rate of ≥25 per 10 HPF had a sensitivity and specificity for predicting a BRCA1 mutation of 81% and 83%, respectively, and 79% and 91%, respectively, by each pathologist. The sensitivity could be increased at the expense of specificity using triple-negative phenotype and/or high mitotic rate. The sensitivity and specificity of this combination was 94% and 74%, respectively, and 87% and 81%, respectively, by each pathologist. This combination did not maintain significance in 59 additional surgical specimens after neoadjuvant chemotherapy, but the combination had a high sensitivity but decreased specificity when only core biopsy specimens were evaluated. The sensitivity and specificity for these were 95% and 57%, respectively, and 95% and 65%, respectively, by each pathologist. No good predictors of BRCA2 mutations were observed.

Conclusions: The combination of triple-negative phenotype and/or mitotic rate ≥25 per 10 HPF in an untreated surgical or core biopsy specimen may improve the selection of high-risk patients for BRCA testing.

252 Relationship between Molecular Subtype of Invasive Breast Carcinoma (IBC) and Expression of Gross Cystic Disease Fluid Protein 15 (GCDFP), Mammaglobin (MGB), and Androgen Receptor (AR)

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Background: IBCs can be subtyped based on gene expression into Luminal (A and B), HER2 and basal-like carcinomas (BLCs). The relative frequency of expression of GCDFP, a marker of apocrine differentiation, has not to our knowledge been assessed in these subtypes. This is particularly relevant since recent studies suggest that the HER2 subtype overlaps with a molecular apocrine subtype which expresses AR and preferentially displays "apocrine morphology," though the latter seems somewhat subjective. In addition, MGB expression has rarely been analyzed in these IBC subtypes.

Design: Tissue microarrays (TMAs) were constructed from paraffin blocks of 71 IBCs and labeled by IHC for ER, PR, HER2, CK5/6 and EGFR to subtype them as Luminal A (ER+ and/or PR+; HER2-), Luminal B (ER+ and/or PR+; HER2+), HER2 (ER, PR-; HER2+), BLC (ER-, PR-, HER2-; CK5/6 and/or EGFR+), or Unclassified Triple Negative Carcinomas (UTNC) (ER, PR, HER2, CK5/6, EGFR-). Five 1mm diameter spots per case were taken. TMA sections were labeled by IHC for GCDFP, AR, and MGB. Any labeling with these markers was considered a positive result, and correlation with subtypes was analyzed.

Results:

GCDFP, MGB and AR Immunolabeling

Cancer Subtype	GCDFP	Mammaglobin	AR
BLC	4.8% (1/21)	24% (5/21)	24% (5/21)
UTNC	0 (0/12)	17% (2/12)	17% (2/12)
Luminal A	65% (11/17)	80% (14/17)	82% (14/17)
Luminal B	71% (5/7)	85% (6/7)	71% (5/7)
HER2	71% (10/14)	71% (10/14)	71% (10/14)

GCDFP, MGB, and AR were less likely to be expressed in BLC than in HER-2 cancers ($p=0.000021$, 0.013, and 0.013 respectively) or Luminal cancers ($p=0.00002$, 0.00008, and 0.0003 respectively). However, the difference in GCDFP, MGB, or AR expression between HER2 and Luminal cancers was not significant ($p=1.0$, 0.671, and 0.671 respectively).

Conclusions: Luminal cancers demonstrate similar degrees of apocrine differentiation, as assessed by GCDFP and AR immunoreactivity, as do HER2 cancers. Most BLC and UNTC are negative for both MGB and GCDFP on our TMAs, which approximate the amount of material obtained by core biopsy. Given the frequent absence of specific

markers of breast origin (ER, PR, GCDFP, MGB) in BLC and UNTC, and their frequent absence of a significant associated in situ component, correlation with clinical findings may be needed to exclude the possibility of a metastasis to the breast when BLC or UTNC are encountered on core biopsy.

253 Computer Aided Measurement of Tumor Area in Positive Lymph Nodes Is an Alternative Way To Predict Prognosis of Metastatic Breast Cancer

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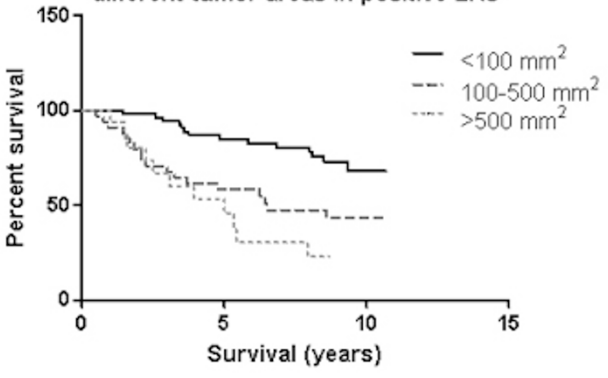
Background: The number of positive lymph nodes (LNs) is the only node-related factor recognized by AJCC for prognostic evaluation of breast cancer. Counting the number of LNs may be problematic in cases of matted LNs. In addition, the number of positive LNs may not completely reflect the degree of tumor involvement. For example, microinvasion or complete tumor replacement in a LN are both counted as one positive LN after the diagnosis of the first macrometastatic LN.

Design: From 1998-2000, 127 patients were diagnosed with breast cancer metastasized to regional LNs in our institution. Areas of metastatic cancer in the LNs were identified and measured using Olympus MicroSuite5 software. Survival and prognosis of patients were compared in relation to the LN tumor area vs. number of positive LNs according to AJCC's classification (N1, N2 and N3).

Results: The patients are divided into 3 groups according to the LN tumor areas: 1) <100mm^2, 2) 100-500mm^2, 3) >500mm^2. Survival analysis of the 3 groups showed statistical difference with a 5-year survival of 75%, 53% and 44%, respectively (Fig 1).

Figure 1: Percent survival of patients with different tumor areas in positive LNs



The survival between N1 (1-3 LNs), N2 (4-9), and N3 (>9) is also different with a 5-year survival of 75%, 52%, and 40% respectively. Interestingly, 15% of the deceased N3 patients showed better prognosis (with <350 mm^2 tumor areas) compared to other N3 patients with mean tumor area of 711mm^2.

Conclusions: Our retrospective study used imaging analysis software to measure tumor areas of positive LNs as an estimation of tumor volume. Our results suggest that LN tumor area is accurate in depicting the extent of metastatic cancer involvement and the prognosis independent of the number of positive LNs. Patients with tumor area <100mm^2 showed better prognosis, and those >500mm^2 showed worst prognosis, with mean survival of 4.9 and 3.4 years, respectively. Although the prognosis is comparable between LN tumor areas and AJCC LN stages, 15% of N3 patients showed better survival due to small tumor areas. Hence, computer aided tumor area analysis can be used as an alternative way to predict prognosis in breast cancer patients.

254 Reevaluation and Identification of the Best Immunohistochemical Panel for Differentiating Breast Ductal Adenocarcinoma from Pancreatic Adenocarcinoma

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Background: When working on an unknown primary, differentiation of adenocarcinoma of the pancreas from ductal carcinoma of the breast can be challenging. Estrogen receptor (ER) is considered as the best marker in this regard; however, only about 70% of ductal carcinoma of the breast may be positive for ER and the positive rate is much lower in high grade ductal carcinomas. Many tumor-associated markers have been reported to be useful in this regard; however, the reproducibility of these markers has not been tested and confirmed in one system. This study investigates the utility of 15 different immunohistochemical markers in the distinction of ductal carcinoma of the breast from pancreatic adenocarcinoma.

Design: We immunohistochemically evaluated the expression of 1) epithelial markers (CK17, CK19, CK903), 2) mucin gene products (MUC1, MUC2, MUC4, MUC5AC, MUC6), 3) tumor suppressor genes and transcription factors (ER, PR, Dpc4/SMAD4), and 4) tumor-associated proteins (KOC, maspin, CA19-9, GCDFP-15) on 70 cases of ductal carcinoma of the breast (40 grade II cases and 30 grade III cases) and 70 cases of pancreatic adenocarcinoma on tissue microarray sections. The staining intensity was graded as weak or strong. The distribution was recorded as negative (<5% of tumor cells stained), 1+ (5-25%), 2+ (26-50%), 3+ (51-75%), or 4+ (>75%).

Results: ER was positive in 75% of grade II ductal carcinomas; but it was only positive in 18% of grade III ductal carcinomas. The immunostaining results are summarized in Table 1.

Table 1. Summary of the Immunohistochemical Staining Results

Markers	Pancreatic Adenocarcinoma (N=70)	Breast Ductal Carcinoma (N=70)
KOC	95%	6%
ER	0	53%
MUC5AC	67%	0
CK17	60%	8.4%
MUC4	50%	0
Dpc4/SMAD4	41%	90%
Maspin	100%	24%
CA19-9	84%	16%
MUC1	95%	95%
MUC2	4%	4%
MUC6	17%	10%
PR	0	29%
GCDFP-15	0	27%
CK903	27%	43%
CK19	75%	73%

Conclusions: Our data demonstrate that KOC, ER, CK17 and MUC5AC are an effective antibody panel in the distinction of pancreatic adenocarcinoma from ductal carcinoma of the breast. In addition, Dpc4/SMAD4, CA19-9, MUC4, and maspin may have some utilities as well.

255 Transmembrane Protein E-Cadherin May Not Be Necessary for Duct Formation in Breast Carcinomas

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Background: Two major forms of breast carcinomas are invasive ductal (IDC) and invasive lobular (ILC) carcinomas. IDC usually show duct formation. ILC typically show single file growth pattern and lack of ductal differentiation. E-cadherin, a transmembrane protein, has been shown to be a useful aid for differentiating between IDC and ILC. E-cadherin is usually negative in ILC but positive in IDC with a strong membranous staining pattern by immunohistology. Thus, E-cadherin protein is thought to play a role in forming the characteristic histologic morphology of breast carcinomas. While studying invasive mammary carcinomas with mixed invasive ductal and lobular features, we have encountered four cases of invasive carcinomas with E-cadherin negative, duct forming areas.

Design: Invasive mammary carcinomas with mixed ductal and lobular features based on histomorphology were identified in our department file. E-cadherin stain was performed either during the initial pathologic examination or during the current study.

Results: We identified 51 cases of invasive mammary carcinomas with mixed ductal and lobular features based on histomorphology in our department file from 2005 to 2009. E-cadherin stain showed that 8 cases are negative for E-cadherin and therefore the 8 cases were re-classified as ILC. These 8 cases showed tumor cells predominately growing in single file or cords like pattern. Interestingly, we also noticed that tumor cells focally formed ducts in 4 of the 8 cases (well-formed ducts in 1 case; poorly formed ducts in three). In spite of the duct formation, the E-cadherin negativity and dominant single file or cords like growth pattern favor a diagnosis of ILC.

Conclusions: In a small proportion of ILC, E-cadherin negative tumor cells may form duct like structures. Thus, E-cadherin protein may not be necessary for duct formation in breast cancers. The finding may facilitate further study on the biological role of adhesion and cell polarity related molecules in breast cancers.

256 Intraoperative Detection of Sentinel Lymph Node Metastases from Breast Cancer: Does the Pathologist Experience, Subspecialty or the Surgeon Experience Matter?

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Background: The purpose of this study was to evaluate the surgeons' and pathologists' performance in detecting tumor metastases in SLN biopsy.

Design: Intraoperative touch preparation (TP) or frozen section (FS) was performed on SLN of consecutive breast cancer patients from 2007 to 2009. Pathologists were divided into three groups, junior ≤5 years, middle 6 to 10 years and senior ≥11 years; cytologists versus non-cytologists; and breast pathologists versus non-breast pathologists. Sensitivity and specificity of detecting positive SLN were recorded for individual pathologists and subgroups. Surgeons were divided into 2 groups, junior ≤5 years and senior ≥11 years. Total number of SLNs and order of positive SLN were recorded. Fisher's exact test and Wilcoxon rank sum test were used for statistical analyses.

Results: There were 396 patients with SLN biopsy, 124 (31.3%) patients had at least one positive SLN. While FS was performed in 73 patients, TP was performed in 323 patients. Sensitivity was 89.4% for FS and 54.5% for TP. For the pathologists, there were 5 junior, 3 middle and 4 senior; 3 cytologists and 9 non-cytologists; and 3 breast pathologists and 9 non-breast pathologists. There was no statistically significant difference in pathologists' performance in relation to subspecialty. False negative rate (FNR) for cytologists vs. non-cytologists was 11 of 30 (36.7%) vs. 29 of 94 (30.9%) respectively. Breast pathologist vs. non-breast pathologist FNR was 9 of 21 (42.9%) vs. 31 of 103 (30.1%) respectively. There were 2 senior and 2 junior surgeons. There was no statistically significant difference between surgeons' performance with regard to the order of positive SLN. While positive SLN order was either #1 or #2 in 55 of 58 (94.8%) patients for senior surgeons, it was 63 of 69 patients (91.3%) for junior surgeon. All positive SLNs were #6 or below. Junior surgeons tended to have higher number of SLNs per patient than senior surgeons with median and range of 3 (1 to 6) and 3 (1 to 12) respectively (p<.0001).

Conclusions: Regardless of pathologist's experience or subspecialty, the FNR for SLN is similar and dictated by the metastases size. We recommend performing SLN biopsy for a maximum of 6 lymph nodes.

257 Using Image Analysis as a Tool for Evaluation of Prognostic and Predictive Biomarkers of Breast Cancer: How Reliable Is It?

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Background: Estrogen receptor (ER), Progesterone receptor (PR) and HER2 are important prognostic and predictive biomarkers for breast cancers and are routinely tested by immunohistochemical (IHC) stain. The accuracy of these test results has huge impact on the patients' management. A critical factor that contributes to the result is the scoring of IHC. This study investigates how computerized image analysis can play a role in reliable scoring, and identifies potential pitfalls with common methods.

Design: 34 cases of invasive ductal carcinoma were evaluated (10 ER and 24 HER2). These stains were manually scored by a pathologist and the HER2 results were confirmed by FISH. For ER, the percent of nuclear reactivity and staining intensity was scored. For HER2, the percentage of membranous reactivity, completeness of membranous reactivity and staining intensity was recorded according to CAP standards. These stains were then blindly scored by multiple commercially available image analysis algorithms from Definiens (Munich, Germany) and Aperio Technologies (Vista, CA). Each algorithm was customized specifically for each biomarker and tissue. The results were compared with the semi-quantitative manual scoring, which was considered the gold standard in this study.

Results: For HER2 positive group, algorithm 2 scored 5/5 cases within the positive range, while algorithm 1 scored 1/5 cases out of positive range. For HER2 negative group, both algorithms scored 10/10 case within the negative range. For HER2 equivocal group, 9/9 cases were scored within the equivocal group. For ER, both algorithms scored 1/10 cases out of range. The performance of algorithm 2 more resembled manual scoring by pathologist than algorithm 1. The key advantage of the algorithms was reproducibility.

Conclusions: Commercially available computerized image analysis can be useful in evaluation of ER and HER2 IHC results. Quality assurance of image analysis by pathologists is warranted. Image analysis should only be used as adjunct to pathologist's evaluation.

258 Columnar Cell Lesions of the Breast: Association with Other Types of Fibrocystic Change and Distribution in Hispanic and Non-Hispanic White Women

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Background: Columnar cell lesions encompass a spectrum including columnar cell change, columnar cell hyperplasia, flat epithelial atypia and micropapillary DCIS. Known for many years under different names, this group of lesions is linked by a similar cytomorphology and reactivity pattern to HMW keratins. Relatively few studies have examined the association of columnar cell lesions with ethnicity and with other types of fibrocystic change. We evaluated 286 breast benign biopsies that were initially diagnosed as non-proliferative, proliferative without atypia, or atypical hyperplasia to review the frequency of columnar cell lesions and association with other fibrocystic changes.

Design: Three pathologists reviewed 286 breast biopsies culled from 14,792 women with benign breast biopsy diagnoses rendered in 1996-2007 in New Mexico. Five year follow-up was available. Thirty parameters were evaluated histologically and entered into an Access database. The pathologists used criteria of Schnitt and Collins to classify fibrocystic changes including columnar cell lesions. Ethnicity and patient age were obtained. Statistical analysis was completed by a statistician. The pathologists were blinded to patient outcome, and interobserver variability was assessed.

Results: Age ranged from 30 to 87 (mean of 55 years). Of the 149 white Non-Hispanic women selected for the study, 66 (44%) had columnar lesions, and of the 58 Hispanic women, 29 (50%) had columnar lesions (79 patients had unknown ethnicity). Columnar cell changes were unifocal in 36 percent and multifocal (more than two blocks positive) in 45% of patients. Columnar cell hyperplasia was more often multifocal than columnar cell change. Two patients had flat epithelial atypia. For the period 1996-2007 in this cohort, there were no significant differences in frequency of columnar cell lesions in Non-Hispanic and Hispanic white women. Chi-square analysis was performed and showed a statistically significant positive association of columnar cell lesions with usual ductal hyperplasia, sclerosing adenosis, and apocrine metaplasia (p value < 0.0001).

Conclusions: In this initial study, the incidence of columnar cell lesions appeared similar in recently diagnosed Hispanic and Non-Hispanic white women. A strong positive association was noted between columnar cell lesions and other specific fibrocystic changes. The large number of columnar cell change and hyperplasia contrasted with a rare diagnosis of flat epithelial atypia.

259 Cortactin Gene Amplification and Expression in Breast Cancer

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Background: 11q13 amplification is found in up to 15% of breast cancers. Multiple genes, including cyclin D1, map to the smallest region of amplification of 11q13. Out of these genes, cortactin (*CTTN*) has been shown to be consistently overexpressed at the mRNA level in tumours harbouring 11q13 amplification. The aims of this study were twofold: to determine whether CTTN is overexpressed at the protein level in tumours harbouring 11q13 amplification and to define the correlations between cortactin overexpression and gene amplification with clinicopathological features of breast cancers and survival of breast cancer patients.

Design: A cohort of 245 patients with invasive breast cancer treated with therapeutic surgery followed by adjuvant anthracycline-based chemotherapy were included in a tissue microarray containing two replicate cores. Immunohistochemistry was performed with a monoclonal antibody against CTTN and semi-quantitatively assessed using the 'quick score' system (intensity 0-3 x distribution 0-6), with observers blinded to 11q13 status and clinicopathological features. CTTN expression levels were classified as low <5, intermediate 5-10, high ≥12. Chromogenic *in situ* hybridisation (CISH) with probes mapping to the 11q13 smallest region of amplification was performed with observers blinded to CTTN expression levels and clinicopathological features. Amplification was defined as >5 signals per nucleus or large gene copy clusters in more than 50% of cancer cells.

Results: Amplification of 11q13 was observed in 12% of cases. High CTTN expression was found in 11% of cases. A strong correlation between 11q13 amplification and CTTN expression levels was found (Mann-Whitney U test, $p<0.00001$). Out of the 27 cases harbouring 11q13 amplification, only 1 had CTTN expression levels < 5. After p value adjustment for multiple comparisons, neither CTTN expression levels nor 11q13 amplification showed significant associations with clinicopathological features. Although both cyclin D1 and CTTN were significantly expressed at higher levels in cases harbouring 11q13 amplification, no correlation between cyclin D1 and CTTN expression levels was found. CTTN expression and 11q13 amplification were not associated with survival.

Conclusions: CTTN is expressed at higher levels in breast cancers harbouring 11q13 amplification, suggesting that CTTN may also be one of the drivers of this amplicon. However CTTN expression is not associated with the outcome of breast cancer patients treated with anthracycline-based chemotherapy.

260 Estrogen Receptor (ER) and Progesterone Receptor (PR) Immunohistochemistry (IHC) Results in Breast Carcinoma Using Varying Fixation Times in Different Fixatives

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Background: Accurate determination of ER and PR status in breast carcinoma is essential because their presence warrants hormonal therapy. The high levels of discordance in Her-2/neu and ER test results reported in the literature (up to 20% and 30%, respectively) are thought to be partly due to variation in pre-analytic factors. To address this, ASCO/CAP made recommendations to normalize fixation for breast biomarker IHC, stating that breast core needle biopsies (CNB) should be fixed >6 and <48 hours (hrs) and excisional biopsies >8 and <72 hrs in 10% formalin.

Design: A mastectomy specimen with a 4 cm known invasive lobular carcinoma (ER+, PR+ and Her-2/neu-) underwent routine clinical sampling and breast biomarker IHC as per ASCO/CAP guidelines: 10 hr fixation in 10% formalin. The remaining tumor was stored fresh at 4°C for 4 days and subsequently cut into 92 CNB-sized pieces (0.5-1.5 cm in length and 0.2 cm in diameter). Each piece was fixed in 20 mL of 10% formalin for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 48, 72, or 168 hrs or 20 mL of Pen-Fix, Bouin's solution, Sakura molecular fixative, zinc formalin, or 15% formalin at similar intervals (only Pen-Fix for 168 hrs). Samples were processed on the Sakura Tissue-Tek VIP tissue processor with no additional fixation. One H&E slide was made for each block. IHC was performed with the 6F11 ER and 636 PR antibody clones with appropriate controls. Two pathologists independently interpreted the IHC blindly, documenting the intensity and percentage of tumor ER and PR staining.

Results: The patient's tumor showed >95% of tumor cells with 3+ ER and PR IHC staining in the clinical sample. All 92 blocks had invasive tumor, except one, which had lobular carcinoma in situ (LCIS), on which IHC was interpreted. After 4 days at 4°C, the tumor showed no degradation and no differences in ER and PR staining for all samples fixed in 10% formalin. In fact, there was no significant difference in ER and PR staining for 15% formalin, zinc formalin, Pen-Fix, Sakura molecular fixative, and most of the Bouin's-fixed samples. Only two Bouin's-fixed samples (48, 72 hrs) had a significant decrease in ER staining, which was still interpreted as positive. No effect was seen with PR.

Conclusions: In our study, the pre-analytical variables of fixative type and fixation time, ranging from 1 hr to 1 week, did not affect the accuracy of ER and PR IHC results in CNB-sized tumor sections.

261 Prognostic Value of Multiple Triple-Negative Markers in Breast Cancer

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Background: Triple-negative (TN) tumors are a subset of breast cancer (BC) that lack estrogen and progesterone receptors and express low amounts of HER2. They have been found to be associated with a worse prognosis. We have previously reported four biomarkers including integrin β4, Sox9 (SRY box-9), IMP3 (IGF-II mRNA binding protein-3), and a cytoskeleton protein fascin that are significantly associated with TN BC and expression of basal cytokeratin CK5/6. Furthermore, while each of them shows a tendency towards worse prognosis the difference is not statistically significant. In this retrospective study we evaluated various combinations of these four TN markers in BC to see if expression of more than one marker together is associated with worse overall prognosis.

Design: The study group comprised of 189 cases of invasive ductal BC retrieved from the files over a 10-year period. Tumor characteristics including CK5/6, ER, PR, and HER2 status were obtained from pathology reports. IHC was performed on paraffin-embedded tissue using a rabbit PAb against Sox9, mouse MAbs against IMP3 and fascin, and a rat MAb against integrin β4. Overall survival data were available in 138 cases, of these 23% were TN. Statistical analysis was performed using JMP 8.0 (SAS Institute).

Results: All four markers are associated with TN status and a positive CK5/6 expression with statistical significance. Combination of these markers provides better predictions than any marker used alone. A total of 10 patients with quadruple positive BC had significant worse overall survival than the patients with BC not expressing at least one of these markers (P=0.0033, Kaplan-Meier log-rank test), so did 14 patients with Sox9,

β4 and fascin triple positive BC (P=0.0052), and so did 17 patients with β4 and fascin double positive BC (P=0.0021). The proportional hazard analysis showed hazard ratios of 2.98 for double positivity of β4 and fascin (P=0.03).

Conclusions: Of the four markers we evaluated, combination of integrin β4 and fascin provides the best prediction for worse prognosis and is associated with the highest hazard ratio. Given their close locations within the cell, integrin [beta4] and fascin could work synergistically in the same molecular machinery or in the same pathway. TN BC is a heterogenous group and individual tumor could undergo different progression pathway.

262 Myoepithelial Marker Expression in Breast Core Biopsy High Grade DCIS as a Predictor of Invasive Carcinoma in Subsequent Resection Specimens

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Background: High-grade ductal carcinoma in-situ (DCIS) is a major risk factor in the development of invasive ductal carcinoma. Immunohistochemical (IHC) demonstration of myoepithelial cells provides convincing evidence against an invasive lesion. We analyzed a set of myoepithelial markers in core biopsies with only high grade DCIS to determine the staining pattern and correlation with followup excision.

Design: Thirty five cases of high grade DCIS in core biopsies with follow up excision were selected for a period between 2004 to 2009. All core biopsies were reviewed by two authors and high grade DCIS was confirmed. Myoepithelial markers [p63, calponin, smooth muscle myosin heavy chain (SMMHC), smooth muscle actin (SMA)] were immunostained and evaluated. Stain intensity (1 = weak; 2 = intermediate and 3 = strong), % partial absence of myoepithelium, and % of myoepithelial attenuation were scored for p63, calponin, and SMMHS. For SMA, only stain intensity was graded. We arbitrary established a threshold of > 20% absence of myoepithelium to be significant for all groups.

Results: Of the 35 cases, 7 (20%), had follow up invasive ductal carcinoma (Group 1) and 28 (80%) showed residual DCIS without invasion or no residual DCIS (Group 2). In group I, calponin (83%), p63 (86%), SMMHS (67%), showed significant partial absence of these three markers. In group 2, calponin (33%), p63 (54%), and SMMHS (48%) showed significant partial absence of these markers. There is no difference for % of attenuation between group1 vs group 2 (calponin- 85% vs 89%; p63- 100% vs 100%; SMMHS- 90% vs 97%). Both groups show decrease stain intensity for all the myoepithelial markers (compared with normal internal control), however no significant difference of stain intensity was observed between group1 vs group 2 (calponin- 100% vs 86%; p63- 85% vs 89%; SMMHS- 86% vs 89%; SMA- 83% vs 79%).

Conclusions: Our results show decreased stain intensity and attenuation of all myoepithelial markers in high grade DCIS. Most of the cases showed different degrees of partial absence of p63, calponin, and SMMHS. The percentage of partial absence of myoepithelium appears increased in cases with follow up invasive ductal carcinoma (Group 1). The biologic significance of this absence remains to be determined. However, our observations suggest that it may be taken into consideration for prediction of invasive carcinoma on follow-up excision.

263 Oligonucleotide Array Analysis of the *HER2* Amplicon in Breast Cancers

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Background: *HER2* amplification is associated with poor prognosis, yet targeted therapy moderates the prognosis of some patients with amplified tumors. Prior studies have shown that breast cancers often have a complex pattern of genetic abnormalities, and that *HER2* amplification is rarely the only gene that is amplified. Several other genes of interest co-localize with *HER2* on chromosome 17, including StAR-related lipid transfer (*STARD3*), Growth Factor Receptor Bound Protein 7 (*GRB7*), Retinoic Acid Receptor Alpha (*RARA*), and DNA Topoisomerase II alpha (*TOP2A*). We undertook an analysis of the *HER2* amplicon by oligonucleotide array in *HER2* gene amplified tumors, with attention to these co-localized genes. The centromere of chromosome 17 was also analyzed, because commercially available FISH assays for *HER2* typically include a probe to centromere 17 (CEP 17, D17Z1).

Design: Initial HER2 studies on breast cancers were performed by immunohistochemisty and FISH. Fourteen HER2 positive cancers were selected which had fresh frozen tissue available for assay by Agilent (Santa Clara, CA, USA) human genome CGH microarray 105A. Analysis was performed using Agilent (Santa Clara, CA, USA) genomic workbench 5.0 software. Loci evaluated included the centromere region (chr17:22,615,505-22,644,166), *STARD3* (chr17:35,062,877-35,073,250), *HER2* (chr17:35,109,780-35,138,441), *GRB7* (chr17:35,149,746-35,157,064), *RARA* (chr17:35,751,868-35,767,420), and *TOP2A* (chr17:35,798,322-35,827,695). The gene copy number was noted as loss (-0.2 to <-0.7), normal (< 0.2 to >-0.2), gain (0.2 to < 0.5), or high copy gain (> 0.5).

Results: All 14 breast tumors exhibited high copy number gain by oligonucleotide array in the *HER2* locus. In addition, all cases showed co-amplification of the *STARD3* and *GRB7* genes. In contrast, *RARA* and *TOP2A* showed co-amplification in only in 2 cases each and loss in 4 cases each. The centromere region of chromosome 17 showed a gain in copy number in 4 cases (including 1 high copy number gain), loss in 3 cases, and was normal in 7 cases.

HER2 amplicon, gene copy number changes

	HER2	*STARD3*	*GRB7*	*RARA*	*TOP2A*	*Centromere*
Gain	14	14	14	2	2	4
Normal	0	0	0	8	8	7
Loss	0	0	0	4	4	3

Number of cases listed

Conclusions: By oligonucleotide array, *GRB7* and *STARD3* co-amplify with *HER2* in breast cancers, but rarely *RARA* or *TOP2A*. In *HER2* amplified breast cancers, the centromere not infrequently shows gains in copy number. The 4 cases with centromere gain did not exhibit whole chromosome polysomy.

264 Luminal A Versus Luminal B Tumors: Significance of Proliferation Index in ER Positive HER2 Negative Tumors

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Background: According to gene expression profiling studies, luminal tumors are divided into two groups with distinctly different prognosis. Luminal A (LUMA) tumors have significantly better prognosis than luminal B (LUMB) tumors. The identification of these 2 groups based on immunohistochemistry (IHC) is currently a contentious issue. Several IHC based definitions exist, including the most recently proposed Ki-67 labeling index (LI) cut-off of 14% to distinguish between LUMA and LUMB tumors (JNCI.2009;101:736-750).

Design: In order to examine the validity of several definitions, we studied 56 ER positive, HER2 negative tumors using IHC. These cases were part of a database of tumors subjected to neoadjuvant chemotherapy. All patients have previously received hormonal and chemotherapy. Following pathology parameters were evaluated with respect to tumor recurrence: overall tumor grade, mitosis score, Ki-67 LI (≥14% {high} versus < 14% {low}), progesterone receptor (PR) status, and positivity for androgen receptor (AR) and FOXA1. AR was studied as some studies have suggested relatively good prognosis for AR positive tumors.

Results: Eight patients had recurred and 48 patients were free of tumor at an average follow up period of 47 months. There was no significant difference between tumor size, age and stage between patients who recurred versus those that were free of tumor. Compared to patients that were free of disease, the patients that recurred had a significantly higher Ki-67 labeling index and showed lack of AR. Other variables were not statistically significant.

Table 1

	Recurred (n=8)	Free of tumor (n=48)	P value
Overall tumor grade	Grade 1: 0	Grade 1: 8	0.406
	Grade 2: 7	Grade 2: 37	
	Grade 3: 1	Grade 3: 3	
Mitosis score	Score 1: 6	Score 1: 40	0.709
	Score 2: 2	Score 2: 7	
	Score 3: 0	Score 3: 1	
Ki-67 LI (n=50)	Low: 1	Low: 29	0.003
	High: 7	High: 13	
	Average: 31	Average: 15	
PR status (n=56)	Neg: 2	Neg: 6	0.35
	Pos: 6	Pos:42	
AR status (n=49)	Neg: 3	Neg: 4	0.04
	Pos: 5	Pos: 37	
FOXA1 status (n=47)	Neg: 0	Neg: 0	NA
	Pos:8	Pos:39	

NA: Not applicable

Conclusions: This is a small study with limited average follow up information; however the results suggest that following information in ER positive tumors may be of value in predicting prognosis: level of proliferation activity as measured by Ki-67 labeling index, and AR status. FOXA1 is almost always intensely reactive in all ER positive tumors and has limited value in predicting prognosis.

265 Triple Positive Breast Carcinomas: Is Onco*Type* Dx™ Test Justified?

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Background: As part of our laboratory program of continuous quality assurance (QA) for monitoring predictive and prognostic marker results for breast tumors, we review our metrics for hormone receptors (HR), HER-2/neu testing and correlate them with results of send-out tests, specifically, the onco*type* Dx™ Recurrence Score (RS). This data affords an opportunity to assess metrics for breast cancer subclasses.

Design: Our QA coordinator collects all estrogen/progesterone receptors (ER and PR) and HER2 results, from breast cancer pathology reports. HR are reported according to the Histologic Score (H Score), with a range of 0 (negative results) to 300 (maximum positive result, 100% cells stain 3+). HER2 testing is reported according to recent CAP-ASCO guidelines using immunohistochemistry (IHC) and FISH for IHC 2+ cases. The results of the onco*type* Dx™ test are also collected. These results are used to compare pathologist's performance with tumor grading and interpretation of HR and HER2 results. This was a focused study on ER+/PR+/HER2+ breast carcinomas termed as "Triple Positive" (TP) tumors.

Results: Of the 76 TP tumors available in QA files from 2004-09, 40 tumors were HER2 IHC 3+(73% of these had high, 15% intermediate, 12% low RS); 17 tumors were IHC 2+/FISH amplified (41% of these had high, 12% intermediate, 47% low RS); 19 tumors were 2+/equivocal FISH (11% had high, 63% intermediate, 26% low RS). The mean ER/PR H Scores for these groups were 195/77, 277/87, 261/167 respectively, with a significant difference in ER H scores between the HER2 IHC3+ versus IHC2+ groups. The PR scores were significantly different between the HER2 IHC 3+ and amplified groups versus FISH equivocal group. There was a preponderance of high RS scores in high grade tumors compared to lower grade tumors, probably reflecting lower mitotic activity in lower grade tumors, and higher ER/PR content for HER2 IHC 2+/FISH equivocal tumors.

Conclusions: (1) TP tumors show heterogeneity in hormone receptor content and degree of HER2 amplification. (2) IHC 3+ tumors have the lowest ER & PR content, while IHC 2+/FISH equivocal cases have highest ER & PR content. (3) Predictably, RS are highest with highest HER2 content/lowest ER & PR content and lowest with lowest

HER2 content and higher ER & PR content. (4) Dual endocrine & anti-HER2 therapy (which is often used in combination with chemotherapy) is a mainstay for these patients making it unlikely that onco*type* Dx™ test will add value to therapeutic decisions.

266 Pathologic Features and Distribution of Molecular Phenotype among Young Women with Breast Cancer: Results from the Young Women's Breast Cancer Study

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Background: Prior studies have suggested a higher prevalence of high grade, ER-negative, HER2+, and basal-like carcinomas in young women with breast cancer. However, studies are limited by small numbers and the distribution of poor prognostic features in young women remains unclear. We examined pathologic features and distribution of molecular phenotype in relation to patient age in a large group of young women with invasive breast cancer.

Design: The Young Women's Breast Cancer Study is a multi-center prospective cohort enrolling women (≤40yrs) with newly diagnosed breast cancers. Medical records were reviewed for clinical characteristics, tumor stage and receptor status. Pathologic features were examined by central review. Tumor grade and biomarker expression were used to classify cancers into molecular phenotype.

Results: The distribution of molecular phenotype and pathologic features by age group for the first 287 women reviewed is shown below. In chi-square analyses (not controlled for multiple testing), the only feature that varied significantly by age group was presence of tumor necrosis (p=0.004). There were no significant differences in molecular phenotype, tumor stage or tumor grade. Compared to published results for all breast cancers, a greater proportion of young women had luminal B (36% vs. 10-25%) tumors, and a lesser proportion had luminal A (27% vs. 60-70%).

Clinico-pathologic Feature/Phenotype	<30 years n=31 (%)	31-35 years n=80 (%)	36-40 years n=176 (%)
Luminal A (ER/PR+, HER2-, low or intermediate grade)	9 (29)	22 (28)	47 (27)
Luminal B (ER/PR+ and HER2+ or ER/PR+, HER2- and high grade)	11 (35)	32 (40)	59 (34)
HER2 (ER-, PR-, HER2+)	3 (10)	11 (14)	19 (11)
Triple negative	8 (26)	14 (18)	41 (23)
Tumor grade 3	21 (68)	52 (65)	102 (58)
Tumor necrosis present	15 (48)	14 (18)	39 (22)
Prominent lymphocytic infiltrate	9 (29)	15 (19)	47 (27)
Pushing tumor margins	12 (39)	25 (31)	46 (26)

Conclusions: In our large prospective cohort of young women, clinico-pathologic features and molecular phenotype were similar across age groups. However, this population of young women presented with a different distribution of molecular phenotypes compared to the general population. These findings may have implications with regard to the etiology and prognosis of breast cancer in young women.

267 100% Concordance between IHC and FISH HER2 Testing Cannot Be Achieved Using N9831 Cohort

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Background: Accuracy of HER2 testing is critical for selecting patients eligible for targeted anti-HER2 therapy. One aspect of the 2007 CAP/ASCO guideline recommendations for HER2 testing is to standardize HER2 interpretation.

Design: We analyzed HER2 IHC and FISH results in patients enrolled in NCCTG N9831 trial using the interpretation guidelines provided by the testing manufacturers (Dako HercepTest for IHC and Abbott/Vysis PathVysion for FISH) and the 2007 CAP/ASCO guidelines.

Results: A total of 2809 cases with both IHC and FISH HER2 results are included in this analysis. 2467 cases (88%) are IHC 3+ using HercepTest criteria of >10% 3+ staining; 2577 cases (92%) are HER2 amplified using PathVysion criteria of HER2:CEP17 ratio >=2.0. Using the 2007 CAP/ASCO interpretation guidelines, 2367 cases (84%) are HER2 IHC 3+, and 2539 cases (90%) are HER2 amplified. 252 cases (9.0%) are HER2 amplified but showed no or equivocal overexpression. 80 cases (2.8%) showed HER2 overexpression without amplification. 49 cases (1.7%) are HER2 IHC 0 or 1+, but showed HER2 amplification. A concordance of 99% is achieved between IHC and FISH when tumors show 100% of 3+ staining by IHC. The detailed data on concordance between both methods are presented in the table.

Concordance of IHC and FISH (N=2809)

IHC	FISH >=2.0	FISH >2.2
3+> 10%	2377/2467 (96.4%)	2365/2467 (95.9%)
3+>20%	2325/2403 (96.8%)	2317/2403 (96.4%)
3+>30%	2293/2367 (96.9%)	2287/2367 (96.6%)
3+>40%	2205/2252(97.9%)	2202/2252 (97.8%)
3+>50%	2142/2180(98.3%)	2141/2180 (98.2%)
3+>60%	2092/2124 (98.5%)	2091/2124 (98.4%)
3+>70%	1987/2012 (98.8%)	1986/2012 (98.7%)
3+>80%	1827/1845 (99.0%)	1826/1845 (98.9%)
3+>90%	1707/1723(99.1%)	1706/1723 (99.0%)
3+=100%	1706/1722 (99.1%)	1705/1722 (99.0%)

Conclusions: No IHC 3+ percentage predicted 100% FISH amplification. Testing with either one of the methods would exclude small numbers of patients from potential benefits of anti-HER2 therapy. These data do not suggest superiority of either IHC or FISH for determining HER2 status. Clinical outcome data will be discussed.

We gratefully acknowledge support from the Breast Cancer Research Foundation, Genentech, and NIH CA25224 for this study.

268 FOXA1 Is a Prognostic Marker in Breast Cancer – A Validation Study of 4046 Cases

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Background: Forkhead box protein A (FOXA) proteins play major roles in development and differentiation. Recently, FOXA1 has been identified to play a role in controlling nearly 50% of estrogen receptor target genes and has been deemed as a 'pioneer factor'. It has been previously demonstrated in small studies that FOXA1 is a prognostic marker that correlates with Luminal subtype A tumors. The aim of this study was to investigate the precise role of FOXA1 in breast cancer using a large population based cohort.

Design: Expression of FOXA1 was analyzed in a tissue microarray of 4,046 invasive breast cancer cases with a median follow- up of 12.4 years using immunohistochemistry. Nuclear FOXA1 expression was correlated clinico-pathologic variables using methods and cut-off points as described in literature. The entire cohort was divided into training (n=2038) and validation (n=2008) sets to avoid corrections for multiple comparisons.

Results: Variable intensity of FOXA1 expression was noted in the 3581 interpretable tumors: none (10.6%), weak (3.5%), moderate (19.3%) and strong (55.1%). High level of FOXA1 expression (score >3) was seen in 86% of the cases. FOXA1 expression correlated positively with older age, ER, PR, GATA-3, E-cadherin (each p-value < 0.0001) and negatively with tumor size, tumor grade, nodal status, Ki67, HER2 expression and basal subtype (each p-value < 0.0001). Univariate analyses showed smaller tumor size, lower grade, node negative status, absence of LVI, and expression of ER, PR, lack of HER2, and low Ki67 were independent predictors of better overall survival. Luminal A subtype cancers had best overall survival when compared to other subtypes. FOXA1 is a significant predictor of breast cancer specific survival (p=0.012) and locoregional relapse free survival (p=0.0001). It did not correlate with disease free survival (p=0.110) and distant relapse free survival (p=0.147). In those treated with tamoxifen, low FOXA1 expression was associated with poor overall survival (p<0.0001).

Conclusions: FOXA1 expression is a prognostic marker in breast cancer. More importantly, it predicts response to tamoxifen in hormone receptor positive patients.

269 IKBKE, NFkB and HIF1A Activation in TLR-9 Invasive Ductal Carcinomas: Relationship with Epithelio-Mesenchymal Transition

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Background: TLR-9, a pattern recognition receptor (PRR) first identified in innate/adaptative immunity, recognizes motifs of DNA and RNA released by necrotic/apoptotic cells.TLR-9 also mediates dorso-ventral patterning in embryo by Epithelio-Mesenchymal Transition(EMT) mechanisms. Recent studies suggest that neoplastic cells acquire a functional TLR-9 pathway during cancer progression.

Design: mRNA expression of 18 genes of the TLR-9 pathway (*TLR-9, MyD88, TICAM1, TRAF3, TKB1, IRAK4, IKBKE, IRF3, IRF7, TRAF6, IRAK1, IRAK2, TAK1, IKKa, IKKb, NEMO, cREL, NFkB1*), 8 genes implicated in EMT(*TWIST1, ZEB1, FOXC2, HIF1A, GLUT1, CDH1, VIM, ACTA2/SMA)*and *IL1, IL6, IL8, IFN, TNF, STAT1, SOX1, SOX2, SOX9, CD133,* was performed by real time quantitative RT-PCR in forty invasive carcinomas. TLR-9/CD289, IKBKE, ZEB1, FOXC2, E Cadherin, Vimentin, ACTA2/SMA, MMP13 and HIF1 protein expressions were sought by immunohistochemistry(ICH).

Results: Real time RT-PCR revealed high mRNA levels of (i) both TIR-containing adaptor *TRIF/TICAM1* and *MyD88* (ii) downstream signaling pathways complex components including the breast oncogene IKBKE and NFkB1(iii*) IL1, IL6, IL8, IFN, HIF1A, GLUT1* and (iv) 7 EMT genes (*TWIST1, FOXC2, CDH1, VIM, ACTA2, HIF1A, GLUT1*). IHC showed that TLR-9 and IKBKE protein expressions were respectively increased in 23/40 (57%) and 21/40(52%) cases with a diffuse cytoplasmic staining(score 3/4+), particularly in the triple negative carcinomas (TNC). Among TNC, 5/13 (38%) cases presented an intermediate EMT phenotype (E Cadherin +/-, Vimentin+, ZEB1-/+, Twist1+, FOXC2+). High positive correlation was observed between (i) *TLR-9* and *IKBKE* at mRNA and protein levels (p<0.05), (ii) *TLR-9, NFkB1, HIF1A and GLUT1* coexpression ($p<10^{-7}$), (iii)*TLR-9, NFkB1, HIF1A and GLUT1* coexpression and acquisition of an EMT phenotype ($P<10^{-6}$). (i) and (ii) were further obtained from a large series of 371 ductal breast carcinomas.

Conclusions: A functional and activated TLR-9 pathway was identified in invasive breast carcinomas, particularly in the TNC subgroup. Analysis of the downstream molecular actors suggests that neoplastic cells acquire immune, survival and EMT properties through activation of the *IKBKE/NFkB1/HIF1A/GLUT1* pathway.

270 Flat Ductal Intraepithelial Neoplasia 1 (Flat Epithelial Atypia) in Core Needle Biopsy: What Do We Do?

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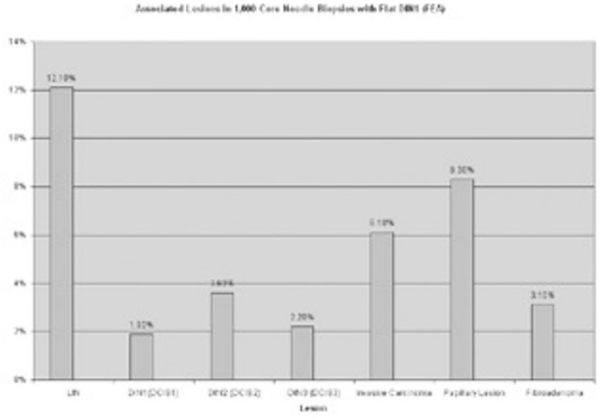
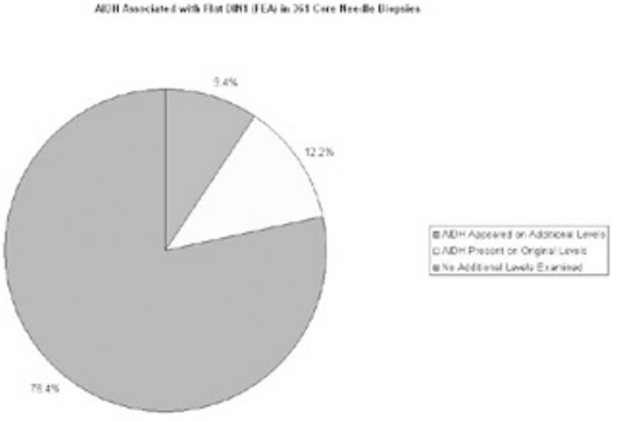
Background: Flat ductal intraepithelial neoplasia 1 (flat DIN1) or flat epithelial atypia (FEA) is a frequent finding in breast core needle biopsies (CNB) particularly those performed for calcifications. The clinical significance and management of flat DIN1(FEA) in CNB is a topic of widespread debate. Our study evaluates the utility of performing additional levels when flat DIN1 (FEA) is present as the most advanced lesion on CNB as proposed previously by one of the authors.

Design: The pathology reports of 1000 CNB at our institution during a 5-year period (2004-2009) included a diagnosis of flat DIN1 (FEA). The presence of concurrent lesions and whether or not levels were examined was noted. Slides from those cases with flat DIN1 (FEA) and AIDH for which levels had been examined were reviewed to determine whether the AIDH was present on the original sections or if it appeared only after evaluation of additional levels.

Results: AIDH was present in 361 (36.1%) of 1000 CNB with flat DIN1 (FEA). Other lesions identified included LIN in 12.1% of the CNB, papillary lesions (in 8.3%), invasive carcinoma (in 6.1%).



Additional levels were examined in 279 of 1000 CNB. Of the 361 CNB with concommitant AIDH levels were examined in 78. AIDH was only present on the additional levels (but not the original levels) in 34 (9.4%) of the CNB.



Examining additional levels when flat DIN1 (FEA) is present as the most advanced lesion on CNB led to approximately a 10% increase in detection of AIDH.

Conclusions: Performing additional levels when flat DIN1 (FEA) is present as the most advanced lesion on CNB leads to a significant increase in detection of lesions warranting an exisional biopsy.

271 Clinicopathologic Characteristics and Molecular Subtypes of Breast Cancers in Young Women

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Background: Breast cancer in young women has been suggested to have distinct biologic characteristics. Gene expression analysis has demonstrated different subtypes of invasive carcinoma with distinct biologic behavior. In this study we analyzed clinico-pathologic characteristics, frequency of different molecular subtypes of invasive carcinoma (based on ER, PR and Her2Neu expression) ploidy, Ki67 and p53 expression in young women.

Design: Women 40 years and younger with a diagnosis of breast carcinoma from 2000 to 2008 were retrieved from the electronic medical record. Clinical and pathologic data such as ethnicity, family history, stage, tumor size, histologic types, and grade were recorded. Tumor markers (ER, PR, Her2Neu, Ki67, ploidy and p53) were performed by immunohistochemistry in a routine fashion and analyzed by automated DAKO ACIS III image analyzer. Positive (3+) and borderline (2+) Her2Neu results were confirmed by FISH.

Results: There were 162 cases with an age range of (21 - 40), mean 34.69. Hispanics comprised 69 (43%); African American, 55 (34%); Caucasian, 32 (20%). Risk factors include: positive family history in 55 (34%), smoking in 37 (29%), use of oral contraceptives in 34 (21%) and age at menarche 12.8, range (9 - 18). There were 34 (21%) stage 1; 57 (35%) stage II; 33 (20%) stage III and 27 (17%) stage IV. Positive lymph nodes were present in 97 (64%). 134 (83%) were invasive ductal carcinoma, 11 (7%) mixed ductal/lobular, 5 (3%) metaplastic, 4 (2%) medullary, 3 (2%) mucinous, 2 (1%) lobular and 7 (4%) in situ. 110 (68%) were grade III; 30 (19%) grade II; 9 (6%) grade I. 108 (72%) were aneuploid and 42 (28%) diploid. Growth fraction (ki67) was > 10% in 130 (87%) and < 10% in 20 (13%). Of the molecular subtypes, ER+PR+Her2- comprised 62 (42%), ER-PR-Her2- 56 (36%), Her2+ in 19 (13%) and ER+PR+Her2+ in 14 (9%). On follow-up, recurrence was noted in 32 (27%), death in 25 (21%) and in 60 (51%) there was no evidence of disease.

Conclusions: Breast cancers in young women of predominantly minority population have poor prognostic features characterized by higher stage at presentation, a predominance of high-grade tumors that were aneuploid with high growth fraction, and unfavorable outcome. In young women the ER+PR+Her2- subtype was the most frequent followed by the triple negative.

272 Integration of Microarray CGH and Expression Analysis Reveals Potential Breast Cancer Therapeutic Targets

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Background: Breast cancer is a heterogeneous disease at the molecular, histopathological and clinical levels. Our aims were to define regions of amplification recurrently amplified in the distinct molecular subtypes of breast cancer (i.e. basal-like, luminal, HER2 and normal breast-like) and to identify genes that are consistently overexpressed when amplified in each molecular subtype.

Design: Two independent cohorts of breast cancer were analysed: i) 69 consecutive frozen invasive breast carcinomas, where samples contained a tumour cell content >70% and ii) 53 frozen, microdissected, grade III invasive ductal carcinomas of no special type (IDCs-NST). Samples from both cohorts were subjected to aCGH using a tiling path 32K BAC array platform and gene expression profiling using the Agilent 25K gene expression arrays (n=69) or the Illumina WG6 v2 platform (n=53). These tumours were classified into luminal, HER2, basal-like and normal-like subgroups based on gene expression profiles. Genes overexpressed when amplified were identified by overlaying aCGH and expression data and performing a multi-Mann-Whitney U test with p values adjusted for multiple comparisons.

Results: In the series of 69 invasive breast cancers, 22% were classified as basal-like, 9% HER2, 68% luminal and 1% normal-like, whereas in the series of 53 IDCs-NST, 26% were classified as basal-like, 26 % HER2, 45% luminal and none as normal breast-like. By overlaying aCGH and gene expression data sets, we identified 4435 genes whose expression was significantly associated with copy number, of which 257 genes were significantly overexpressed when amplified. 116 genes consistently overexpressed when amplified in both datasets include *CCND1, ORAOV1*, *CTTN* on 11q13.2-q13.4 and *PPM1D* and *APPBP2* in 17q23.2. Genes recurrently amplified and significantly overexpressed when amplified in specific subtypes of breast cancer included *ERLIN2, PROSC* and *BRF2* on 8p11.2 in 12% of luminal cancers, *GDI2*, *FBXO18*, and *ATP5C1* on 10p14-10p15 in 18% of basal-like tumours and *WDR68, CCDC44, CCDC47, DDX42, FTSJ3, PSMC5* and *SMARCD2* on 17q23.3 in 26% of HER2 tumours.

Conclusions: Different breast cancer molecular subtypes harbour distinct recurrent amplifications, which encompass genes that may be exploited as therapeutic targets.

273 Preferential Expression of MAD2 in Triple-Negative and High Grade Mammary Carcinoma

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Background: Mitotic Arrest Deficient-Like 2 (MAD2) is part of mitotic spindle assembly checkpoint that prevents the onset of anaphase until all chromosomes are properly aligned at metaphase. Increased expression of MAD2 at mRNA and protein levels has been showed in a number of genetically unstable human neoplasms including breast cancer cell lines. The purpose of this study was to explore the expression of MAD2 in phenotypic subtypes of mammary carcinoma.

Design: Core needle or excisional biopsies from 235 invasive breast cancers were evaluated for the immunohistochemical expression of MAD2, ER, PR, HER2, p63, CK5/6 and HLA-DR. Real-time quantitative PCR for MAD2 was performed on 80 samples.

Results: Thirty-seven carcinomas were classified as either basal-like (based on morphology, triple-negative, and p63/CK5/6 positivity) or medullary (morphology, triple-negative, HLA-DR positivity). Of the 198 ductal carcinomas, 34 were classified as low-grade, 97 as intermediate-grade and 67 as high-grade. A total of 55 ductal carcinomas were triple-negative (7 intermediate-grade, 48 high-grade). All triple-negative ductal and 30 (82%) medullary and basal-like tumors showed nuclear expression of MAD2 by IHC. Similarly, real-time quantitative PCR showed a significantly increased copy number of MAD2 transcript in all high-grade and triple-negative cancers compared to low-grade tumors.

Conclusions: MAD2 expression at the mRNA and protein levels is significantly higher in high-grade breast cancers when compared to low-grade tumors that are more likely to be checkpoint proficient.

274 Drosha and Dicer Down-Regulation in Breast Cancer: A QRT-PCR Analysis

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Background: Non-coding RNAs, in particular miRNAs, play major roles in the development of cancer and global down-regulation of miRNAs has been documented in human cancers. Drosha and Dicer are two key enzymes in the miRNA machinery, whose down-regulation was described in ovarian cancers of aggressive clinical behaviour. Here we investigated the prevalence of Drosha and Dicer down-regulation in breast cancers and their associations with clinicopathological features of the tumours and patients' outcome.

Design: A cohort of 245 patients with invasive breast cancer treated with therapeutic surgery followed by adjuvant anthracycline-based chemotherapy and a series of ten normal breast samples from reduction mammoplasties were retrieved from the histopathology files of the authors' institutions. mRNA was extracted from representative sections of the tumours containing >50% of tumour cells and from sections of normal breast samples enriched for terminal duct-lobular units (>50% of the surface area).

TaqMan quantitative real-time PCR (qRT-PCR) using primers for Drosha and Dicer and for two house keeping genes was performed. Cases were categorised as displaying Drosha or Dicer down-regulation if expression levels were lower than the median expression levels in normal breast tissue and correlated with clinicopathological characteristics of the tumours and patients outcome.

Results: Drosha down-regulation was observed in 18% of cases and was significantly associated with histological grade, high MIB1 labelling index, *HER2* expression and gene amplification, *TOP2A* gene amplification and inversely correlated with BCL2 expression. Dicer down-regulation was found in 46% cases and was significantly associated with histological grade, presence of lympho-vascular invasion, basal markers (EGFR, cytokeratins 5/6 and 17, caveolin 1 and nestin), high MIB1 labelling index and triple negative phenotype and inversely correlated with oestrogen receptor and BCL2 expression. Drosha down-regulation was significantly more prevalent in HER2 positive cancers, whereas the majority of basal-like (76%) and triple negative (78%) cancers displayed Dicer down-regulation. No associations between down-regulation of Drosha and Dicer and outcome were observed.

Conclusions: Drosha and Dicer are preferentially down-regulated in HER2 and basal-like cancers, respectively. Distinct components of the miRNA machinery may be dysfunctional in different subtypes of breast cancer.

275 Human Epidermal Growth Factor Receptor 2 (HER2) Status Determination Using Single Color Versus Dual Color Fluorescence In-Situ Hybridization(FISH) Assay: Impact of Monosomy and Polysomy 17

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Background: The CAP/ASCO guideline is an important document for interpretation of HER2 FISH results. A FISH result of more than 6 HER2 gene copies per nucleus (using single color FISH or S-FISH) or HER2 to chromosome 17 ratio of more than 2.2 (using dual color FISH or D-FISH) are considered positive for HER2 gene amplification. An equivocal result is 4-6 copies of HER2 per cell (S-FISH) or ratio of 1.8 to 2.2 (D-FISH). However, CAP/ASCO guidelines fails to address the discordance that can occur based on the type of probe used (S-FISH versus D-FISH) especially when monosomy or polysomy for chromosome 17 is present.

Design: The current study was performed to determine the impact of monosomy and polysomy 17 in the context of S-FISH and D-FISH. Monosomy 17 was defined as one signal for chromosome enumeration probe 17 (CEP17) in >50% of the examined cells. Polysomy 17 was defined as an average of 3 or more signals for CEP17. The monosomy cases were obtained from our partner institution that performed D-FISH on 53 consultation cases. The 20 polysomy cases were obtained from our previously studied dataset (Mod Pathol. 2009;22 (Supplement 1): Abstract 179). All polysomy cases showed 2+ expression by immunohistochemistry. Although the FISH was performed using dual color probe, the data was analyzed based on HER2 gene signal/cell (S-FISH) versus HER2:CEP17 ratio (D-FISH).

Results:

Monosomy Cases (n=53)

	SFISH- Not Amplified	SFISH- Equivocal	SFISH- Amplified
DFISH- Not Amplified	30	0	0
DFISH- Equivocal	0	0	0
DFISH- Amplified	8*	3*	12

*Discrepant cases (discrepancy rate is 21%)

Polysomy Cases (n=20)

	SFISH- Not Amplified	SFISH-Equivocal	SFISH-Amplified
DFISH- Not Amplified	7	10*	3*
DFISH- Equivocal	0	0	0
DFISH- Amplified	0	0	0

*Discrepant cases (discrepancy rate is 65%)

Conclusions: Invasive breast cancer cases that have monosomy of chromosome 17, D-FISH overestimates HER2 amplification (21%). In contrast, the cases that demonstrate polysomy 17, S-FISH overestimates HER2 gene amplification (65%). Despite some interpretation challenges associated with D-FISH, we still recommend using dual color probe for HER2 FISH. The CAP/ASCO criteria are suboptimal in interpreting these difficult cases. Until a consensus is reached in interpreting these cases, a pragmatic approach should be undertaken to avoid overestimation of HER2 gene amplification.

276 Expression of Stem Cell Marker (ALDH1) Correlates with Aggressive Molecular Subtypes of Breast Cancer

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Background: Data from preclinical models have recently identified ALDH1 (Aldehyde dehydrogenase 1) as a marker of cancer stem cells. The aim of this study was to assess the level of ALDH1 expression within the molecular subtypes of breast cancer that correlates with prognosis and response to adjuvant treatment.

Design: ALDH1 expression was examined in 121 invasive ductal carcinomas (114 primary and 7 locally recurrent tumors). Immunohistochemistry (BD Bioscience, 1:1000) was applied to tissue microarray from 105 cases (arrayed in triplicates) and 16 full section slides of invasive breast carcinoma accessioned between 2007-2009. For the purpose of this study only strong cytoplasmic stain was considered positive while nuclear or weak stain was considered nonspecific. Results were scored in 4-tiered system based on the percentage of positive cells (0= no stain, 1= 1-10%, 2=11-50%, 3=51-100%). Data on tumor characteristics was retrieved from the pathology report. Associations between ALDH1 status, estrogen and progesterone receptors (ER, PR) and HER2/neu status as well as pertinent pathological parameters were assessed with the χ2-test.

Results: There were 75 ER+/HER2/neu-, 23 HER2/neu+ and 23 triple negative (TN) cases. The proportion of tumours expressing ALDH1 in >10% was significantly higher in HER2/neu+ cases compared with ER+/HER2/neu- (39% vs 8% respectively, p=0.0092) and in TN when compared with ER+/HER2/neu- (26% vs 8% respectively, p=0.02). No significance difference was noted between the expression of ALDH1 expression in HER2/neu+ and TN tumors (p=0.34). ALDH1 expression in >10% of the cells in primary tumors did not correlate with patient age, tumour size, Nottingham grade 3, presence of in situ component, lymphovascular invasion or nodal status.

Conclusions: Our study showed that the proportion of tumors that express stem cell marker ALDH1 is significantly higher in aggressive types of breast cancers namely HER2/neu+ and TN subtypes.

277 Flat Epithelial Atypia (FEA) Is a Common Subtype of B3 Breast Lesions and Associated with Non-Invasive Cancer but Not with Invasive Cancer in Final Excision Histology

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Background: The biological behaviour and the optimal management of benign breast lesions with uncertain malignant potential, the so called B3 lesions, found in breast needle core biopsies is still under debate. We addressed this study to compare histological findings in B3 needle core biopsies with final excision specimens to determine associated rates of malignancy.

Design: Consecutive needle core biopsies (NCB) were performed in a three-year period (01.01.2006-31.12.2008). Biopsies were image-guided (31 by ultrasound, 85 stereotactic vacuum-assisted, 6 unknown) for evaluation of breast abnormalities. We reviewed 122 NCB B3 lesions of 91 symptomatic patients and 31 screen-detected women and compared the B3 histological subtypes with the final excision histology.

Results: A total of 1845 NCBs were performed and B3 lesions comprised 6.6% of all B-categories. The most common histological subtype in B3-NCBs was flat epithelia atypia (FEA) in 35.2%, followed by papillary lesions in 21%, and atypical ductal hyperplasia (ADH) in 20%. Reports on excision specimens were available in 66% (81 patients). Final excision histology was benign in 73 (90.2%) and malignant in 8 (9.8%) patients (2 invasive cancer, 6 ductal carcinoma in situ). Of all B3 subtypes, only ADH and FEA were associated with malignancy, whereas only ADH was accompanied by invasive cancer. Of all lesions, FEA was most frequently found in excision specimens (18%).

Conclusions: FEA and ADH are common lesions of the B3-category in needle core biopsies of the breast. Both lesions are associated with malignancy whereas only ADH was related to invasive cancer. Depending on clinical and radiological findings, we conclude that an excision biopsy after diagnosis of FEA is recommended.

278 Expression of Breast Specific Markers in Triple-Negative Breast Carcinomas

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Background: Triple negative (ER-/PR-/HER2-, TN) breast carcinomas are aggressive tumors associated with a high incidence of metastases. However, due to their lack of staining with hormone receptors and HER2, confirmation that metastatic disease originates from a primary TN breast carcinoma can be problematic. Previous studies have evaluated expression of mammaglobin (MGB1), gross cystic disease fluid protein 15 (GCDFP-15), and androgen receptor (AR) in breast carcinomas, however there is limited data that specifically addresses expression of these breast markers in TN breast cancers. In this study, we compared the expression of MGB1, GCDFP-15 and AR in TN, ER+/HER2- (ER+), and HER2+ breast carcinomas and evaluated their efficacy as breast markers for TN tumors.

Design: The expression of MGB1, and GCDFP-15 and AR was evaluated by immunohistochemistry on tissue microarrays containing 108 TN, 44 ER+, and 27 HER2+ primary breast carcinomas. Strong cytoplasmic staining in any tumor cells was scored positive for MGB1 and GCDFP-15, while nuclear staining in more than 1% of tumor cells was considered positive for AR. A Chi-square test was used to compare the expression results between the different tumor groups.

Results: Expression of breast markers in the different tumor groups is summarized in Table 1. At least one of the three markers (MGB1, GCDFP-15, or AR) was detected in 37 (37%) of 100 TN carcinomas compared with 38 (93%) of 41 ER+ carcinomas and 27 (100%) of 27 HER2+ carcinomas.

MGB1, GCDFP-15 and AR Expression in Different Breast Tumors

	MGB1 positivity	GCDFP-15 positivity	AR positivity
TN tumors	21/98 (21%)	17/107 (16%)	20/101 (20%)
ER+ tumors	25/42 (60%)	15/39 (38%)	30/40 (75%)
HER2+ tumors	22/27 (81%)	10/26 (38%)	16/24 (67%)
p value	<0.0001	0.004	<0.0001

Conclusions: MGB1, GCDFP-15, and AR are expressed by TN breast cancers, though less frequently than in ER+ or HER2+ cancers. Never the less, their presence in carcinoma of unknown origin is strongly suggestive of a breast primary even when ER, PR and HER2 are absent.

279 Prognostic Factors in Triple Negative Breast Carcinomas: Is Sub-Classification Using Basal Markers Warranted?

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Background: While most triple negative (ER-/PR-/HER2-, TN) breast carcinomas demonstrate aggressive clinical behavior and high mortality, a subset do not. Although heterogeneity of morphology and biomarkers exist within the TN family of breast cancer, it is not well established whether any such features stratify risk. The subset of basal phenotype TN cancers (defined as CK5/6, CK14 or EGFR+ TN cancers) has received particular attention. In this study we examined whether morphology and biomarkers can distinguish high-risk and low-risk patients in TN breast cancer.

Design: The study population consists of 104 primary TN tumors treated and followed at our institution between 1998-2009 with slides and blocks available from which

a tissue microarray (TMA) was constructed. Pathologic features were reviewed. Biomarker expression was evaluated using immunostaining for androgen receptor, mammaglobin, gross cystic disease fluid protein, CK5/6, CK14, EGFR, Ki67, p53, retinoblastoma protein (Rb) and p16. Statistical analysis was performed using STATA statistical software.

Results: All patients were female with median age at diagnosis of 57 years (range 25-92 years). The group was comprised of 92 ductal cancers, 4 each of metaplastic and adenoid cystic-like cancers, and 2 each of lobular and small cell carcinomas. 41% of cases were node-positive. 79/104 cases (76%) were basal-type based on the above criteria; 16/104 (15%) were non-basal and 9/104 (9%) were uncategorized. Median follow up time for the cohort was 38 months (95% CI 29-47 months). Univariate analysis showed that p16 conferred an improved disease-free survival (DFS), whereas LVI and number of positive lymph nodes were associated with lower likelihood of DFS (OR 4.2, p=0.01 and 1.3, p=0.007 respectively). Basal subtype, age, grade, Ki67 labeling index, and Rb were not predictive of DFS. On multivariate analysis including basal subtype, p16, LVI, number of positive nodes, age, grade, and tumor size, only tumor size and number of positive lymph nodes remained independent predictors of breast cancer-specific survival.

Conclusions: Independent prognostic markers of TN breast cancer are tumor size and nodal status. There is no prognostic value in subclassifying TN cancers using basal markers, Ki67, p53, RB or p16. While the basal phenotype may be of interest from pathogenetic perspective, it does not appear to carry clinical significance.

280 Significance of Lobular Carcinoma In Situ at Margins of Breast Conservation Specimens

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Background: Lobular carcinoma in situ (LCIS) is a known risk factor for development of breast cancer. LCIS at the margin is frequently not reported because margins clear of LCIS are not a surgical goal. There is new emerging evidence of the biological significance of LCIS as possible precursor lesion. At our institution, LCIS with margin assessment is routinely performed as part of pathology report. Our aim is to review the impact of margin status and determine if LCIS found at a margin on breast conservation surgery was associated with an increased incidence of residual/recurrent disease.

Design: We retrospectively reviewed a total of 1334 breast surgical excision specimens at a single institution in a 10 year period. Inclusion criteria for positive group are as follows: primary breast conservation surgery containing invasive and/or in situ carcinoma with LCIS/ALH that had only positive margin with LCIS/ALH. We excluded all invasive carcinoma, DCIS and close margin (less than 1 mm) with LCIS/ALH. We identified 39 cases (2.9%) with LCIS/ALH was solely identified at a margin. A negative control group consisted of 46 cases of invasive carcinoma and/or in-situ carcinoma and clear margins including LCIS/ALH on primary surgical excision. No patients had re-excision. Both groups were matched to tumor type, stage and grade, lymph node status and patient age with clinical follow up in the patient's medical records.

Results: Of the 39 cases: 4 (10%) were lost to follow-up, 12 (30%) had no further procedures performed and 23 (59%) had subsequent pathology specimens which consisted of 11 mastectectomies and 12 re-excisions. Of 23 patients with re-excision, 11 (48%) had residual invasive carcinoma or DCIS, three (13%) had pleomorphic LCIS and five (22%) showed residual classic type LCIS after only LCIS/ALH was identified at a margin. Thirteen (33%) cases had negative residual diseases or no recurrence in their clinical follow up. Follow up months ranges from 1 to 109 months. From the 46 negative control groups, we found three (6.5%) patients with bone or brain metastasis and one local recurrence. Follow up months ranges from 4 to 152 months.

Conclusions: LCIS found at a margin on an excision specimen showed a significant residual and recurrent ipsilateral disease finding when residual invasive carcinoma/DCIS and pleomorphic LCIS were included. Our study showed 36% had significant diseases when compared to 6.5% from negative control groups. Our study supports the view that margin status may play a role in treatment of patients with positive margin with LCIS.

281 Melatonin Receptor in Triple Negative Breast Carcinoma in African-American Women

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Background: The pineal hormone melatonin exerts a regulatory function on cell proliferation and an antiproliferative effect on human tumor cell lines and on the growth of human breast cancer (HBC) cell xenografts in vivo. The membrane bound G-protein coupled M1 melatonin receptor is present in HBC cell lines. Activated mediates the growth suppressive action of melatonin on tumor cells. The M1 receptor was identified in ER and PR positive BC. We studied its presence in triple negative breast cancers (TNBC). Melatonine may be of interest in TNBC with no specific adjuvant treatment available.

Design: Tissue microarrays (TMAs) from TNBC from a 6 year period were studied. Tumors that did not show staining for ER, PR and were scored as 0, 1 or 2 with FISH confirmation for non-amplified HER2, were included. Immunohistochemical (IHC) staining was performed for cytokeratin (CK) 5/6, 7,8,14,18,19, vimentin, CD44, survivin, c-kit, p53. Clinico-pathologic data were included . Statistical analysis was performed using Anova.

Results: Of the 107 African-American (AA) patients, 85 had interpretable tumor on the TMAs. The age range was 23-83, with and average age of 50, of which 46% were younger and 54% older than 50 years of age. 80% of AA patients showed positivity for MR1 MR and 20 % were negative. The positivity of melatonin receptor correlated positively with luminal CK7 expression and negatively with the basal CK 5/6. Statically significant data are presented in Table1.

Table 1

	Variable	Df	F-Test	P Value	Corr Coeff	Corr
All patients	CK5/6	85	2.47343	0.1196	0.17011	Neg.
	CK7	85	7.00917	0.0097*	0.27906	Pos.
	Survivin	85	3.48741	0.0551	0.20883	Neg.
Patients>50 yrs old	CK5/6	49	5.91388	0.0189*	0.33431	Neg.
	CK7	49	8.85167	0.0046*	0.39810	Pos.
	Survivin	49	1.83640	0.1819	0.19392	Neg.

Conclusions: 1. 80% of TNBC in AA are positive for melatonin receptor that may indicate responsiveness to growth inhibition by melatonin. 2. Melatonin receptor expression is negatively correlated with basal CK5/6 expression and positively correlated with luminal cytokeratin 7 expression, reflecting possibly different biologic behavior. 3. Survivin, an inhibitor of apoptosis shows a trend to be negatively correlated with melatonin receptors in all patients younger than 50-years of age.

282 Uroplasminogen Receptor (uPAR) IHC Study in Triple Negative Breast Carcinoma

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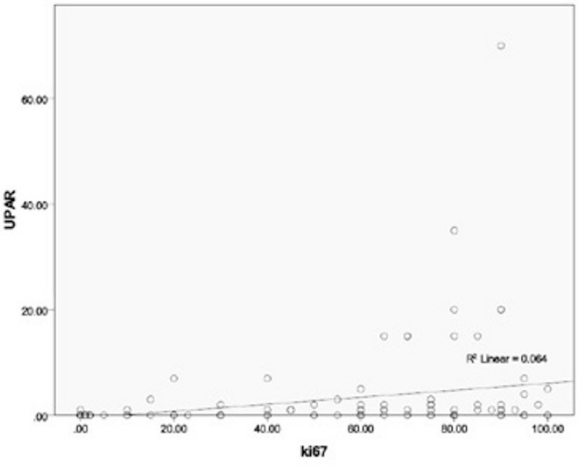
Background: The uroplasminogen activator system is thought to play a role in several different processes important to tumor progression, including tumor growth, angiogenesis and metastasis. UPAR is expressed in most solid tumors and was described as portending worse prognosis in hormone receptors positive breast cancer (BC). The aim of this study is to asses d the presence of uPAR expression in triple negative breast cancers (TNTBC) in comparison with other phenotypic markers and patients' racial background.

Design: Tissue microarrays from 125 BC diagnosed during a 6 year period were studied. Only tumors that did not show staining for ER, PR and were scored as 0, 1 or 2 with FISH confirmation for non-amplified HER2 were included. Immunohistochemical (IHC) staining was performed for cytokeratin (CK) 5/6, 7, 8, 14, 18, 19, vimentin, CD44, survivin, c-kit and p53. Clinical pathologic data included race, age, and TNM staging. Statistical analysis: Pearson correlation coeficient was used, using a 2 tailed p value of .05 as significant.

Results: Our patients included 107 African-American (AA) and 18 Caucasians (CS) women. The age range was 28-83 in AA and 18-80 in CS group, with an average of 50 year old and 40 year old respecively. Overall 41% women were younger than 50 year of age. UPAR staining was observed in 41/90 (46%) of AA and 3/10 (30%) of CS patients. Overall in the four groups we found a positive correlation between the percentage of UPAR and the percenatge of Ki67 staining.



This positive correlation is also present for the AA younger than 50 group (CC of .306, p val < .013), but not observed in the AA women older than 50 year of age. This positive correlation was present in both, the CS younger than 50 yo group (CC .249, p val < .012) and in the CS older than 50 yo group (CC of .233, p val < .023)

Conclusions: 1. UPAR was positively correlated with proliferative rate which is one of the most important prognostic factors for breast cancer. 2. This suggests that targeted anti-UPAT therapy may be beneficial in a subgroup of patients with TNBC, as this group does not benefit form other any specific therapy.

283 Cross-Sectional Study of Breast Cancer among Hispanic Women Living in the United States

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Background: Breast cancer is the most common female malignancy. Differences in incidence and mortality between different racial/ethnic groups were recently reported, but not further explored. Changing demographics in the United States underscore the importance of delineating clinicopathologic variation between racial/ethnic groups.

Design: The 1973-2006 National Cancer Institute, Surveillance, Epidemiology, and End Results (SEER) dataset was used to analyze clinicopathologic differences in primary tumors of the breast between Hispanic and non-Hispanic white women.

Results: Hispanic women with primary tumors of the breast were younger (mean age 56.9 yrs.) than non-Hispanic white women (mean age 62.2 yrs.)(P<0.001), and were more likely to present with advanced stage disease (OR=1.33, P<0.001). Whereas ductal carcinoma was the most common tumor in both ethnic groups (P=0.100), lobular

carcinoma occurred less frequently among Hispanic women (OR=0.86, P<0.001) and medullary (OR=1.40, P<0.001) and inflammatory (OR=1.65, P<0.001) carcinoma occurred more frequently. Hispanic women tended to have tumors with higher-grade morphology (OR=1.16, P<0.001) and were more likely to lack estrogen and progesterone receptor expression (OR=1.32, P<0.001). Multivariate Cox analysis showed Hispanic ethnicity to be a negative independent predictor of survival (HR=1.11, P=0.003).
Conclusions: Hispanic women with breast cancer present at an earlier age and with more advanced stage disease. These data suggest that the initiation of breast cancer screening in Hispanic women at a younger age may help identify a greater number of women with earlier stage disease.

284 The Influence of Insulin-Like Growth Factor 1-Receptor (IGF1R) in Immunophenotypes of Breast Carcinoma

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Background: The biology of breast carcinoma (BC) shows a great variation, reflected by the recent subclassification according to phenotypes based on DNA microarrays or immunohistochemistry (IHC). Currently, there is no data regarding the prevalence of IGF1R in BC subtypes and the impact on the outcome in patients with early BC.
Design: We studied 197 consecutive BC patients in stage I-II treated with conservative surgery and radiation therapy. Phenotypes were assessed based on the expression of ER/PR, HER2, p53, Ki67, CK5/6 and EGFR. Moreover, IGF1R expression (alpha-IGF1R) was evaluated by IHC, IGF1R mRNA by quantitative RT-PCR and IGF1R mutations by direct DNA sequencing. The results were correlated with BC subtypes and patient's outcome.
Results: Patients' average age was 52 years (range 23-88 years) with a median follow-up of 124 months (range 16-251 months). Tumors were classified as luminal A (ER/PR>50%; and p53 or Ki67<20%) in 40% (78/197) cases, luminal B (ER+/-PR<50%, and p53 or Ki67>20%) in 24% (48/197), HER2+ (>30% 3+ or amplified by FISH/CISH) in 19% (37/197) and basal/triple-negative (TN) (ER/PR/HER2- +/-CK5/6+/-EGFR) in 17% (34/197). Luminal A was of low grade (67%), without necrosis (50%), presenting in older patients (43%) as a unilateral mass (42%), of <2 cm (44%) (all p<0.046). IGF1R overexpression was seen more frequently among luminal A (44%) cases, followed by luminal B (28%), HER2+ (21%) and basal/TN (6.6%) (p=0.005), with similar results for mRNA levels (68%, 47%, 40% and 23%, respectively) (p=0.031), but without differences for the mutations (p=ns). Patients with luminal A or B tumors survived longer (94% and 83%, respectively) compared with those with HER2+ (78%) or basal/TN (76%) (p=0.051). Moreover, high IGF1R protein implied better patients' survival among subtypes except for the basal/TN (p=0.013) (Kaplan-Meier; log-rank test). For overall survival, only histologic grade and vascular invasion emerged as significant predictors (p<0.05; Cox regression).
Conclusions: Luminal A is the most frequent subtype of BC in patients in early stage and it is associated with low-risk factors. Moreover, increased IGF1R implies better patient's prognosis for the Luminal A and B and HER2 subtypes, and that might be partially related with its positive cross-talk with ER/PR. Our results highlight the biological and potential clinical relevance of IGF1R in BC subtypes Supported by grant FIS 03/1411and FCVI-HGUA/2006.

285 The Role of EGFR and IGF1R in MAPK and PI3K/Akt Signaling Pathways in HER2-Positive Breast Carcinoma

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Background: The Ras-Raf-MAPK (mitogen-activated protein kinase)-Erk and PI3K/Akt signaling pathways are critical in the regulation of cell growth and survival, respectively. Aberrant activation by growth factors or ligands of tyrosine kinase receptors has been reported in human malignancies. The aim of our study was to analyze the role of EGFR (epidermal growth factor receptor) and IGF1R (Insulin-like growth factor 1 receptor) in the activation of the MAPK Erk1/2 and PI3/Akt pathways in a series of HER2-positive breast carcinoma (BC).
Design: 257 patients with HER2-positive BC, treated at the University General Hospital of Alicante, were included in this study. FISH and/or CISH did confirmation of HER2 status in all 2+ and <30% 3+ tumors. We examined immunohistochemically phosphorylation of p44/42 MAPK (Thr202/Tyr204), Akt (Ser473) and p110 (alpha), and expression of ER, EGFR and IGF1R (alpha). Aberrant expression was correlated with tumor characteristics and outcome.
Results: Patients' mean age was 55 years (SD+/-14 years) and mean follow-up was 71 months (SD+/-48 months); 25.3% patients received trastuzumab combined with other treatment modalities. Tumors were predominantly >2cm (56%), of ductal type (96%), grade 3 (68%), with necrosis (53%) and vascular invasion (55%). ER was positive in 52% cases, increased IGF1R in 42%, EGFR in 16%, p110 in 51%, pAkt in 45% and pMAPK in 21% cases. Tumors with EGFR overexpression were larger than 2cm (p=0.038), of high grade (p=0.024), with vascular invasion (p=0.12), positive lymph nodes (p=0.021), ER-negative (p<0.000) and increased p110/pAkt (p=0.006), but no correlation was found with MAPK phosphorylation status (p=ns). On the other hand, those with IGF1R overexpression were <2cm (p=0.02), without necrosis (p=0.021) or vascular invasion (p=0.01), negative lymph-nodes (p=0.16), ER-positive (p=0.01), and coexpressed p110/pAKT (p=0.021) or pMAPK (p=0.07). However, only a trend towards a better disease-free survival was seen for patients with EGFR-negative tumors (69% vs 61%; p=0.13).
Conclusions: EGFR is expressed in tumors with poor prognostic factors and activation of PI3K/Akt signaling. In contrast, IGF1R correlates with good prognostic factors and activation of both pathways. Our data indicate that inhibition of the MAPK and/or PI3K/Akt pathways targeting EGFR or IGF1R is a promising treatment strategy in a subgroup of HER2-positive BC patients.

286 Role of Transcription Factors [FOXA1, GATA-3] in Predicting Outcome in (ER+) and (ER-) Ductal Carcinoma-In-Situ (DCIS) Patients with and without Invasive Carcinoma (IC): A Retrospective Subset Analysis

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Background: Estrogen receptor (ER) plays key role in development and influence of treatment outcome in breast cancer patients. The two broad groups of invasive carcinoma (IC) based on ER expression, namely ER(+) and ER(-), are yet to be fully understood in DCIS. Absence of ER has been indicated as a predictor of IC recurrence in DCIS. Recently functional interaction between FOXA1 transcription factor and ER has been shown to play a critical role in suppressing ER-dependent breast cancer cell growth and tumorigenesis in vivo. The GATA family of transcription factors, highly expressed in the mammary epithelium, has emerged as a strong predictor of tumor differentiation, ER status, and clinical outcome. The specific aim of this study is to analyze the expression of novel biological transcription markers, FOXA1, GATA-3, and established proliferation markers such as MIB-1(Ki-67); and HER2/neu in ER(+) and ER(-) DCIS patients.
Design: Two hundred and ninty-one cases of DCIS were retrieved from our Pathology database, with complete data on 219 cases. The follow-up period from 1988-2009. Recurrence is defined in terms of DCIS or IC. A 10% or more nuclear staining of the tumor cells was considered positive for FOXA1, GATA3, ER, PR. MIB-1 proliferation index (1-10%-low, 11-25%-intermediate, 26-50%-high, >51%-very high); HER-2 is a membrane stain and interpreted as per routine guidelines for IC. *Statistical analysis:* Fisher's exact test for testing the dependence between clinical outcome (non-recurrence, recurrent-IDC and recurrent -DCIS) for each biomarker with calculation of p-value.
Results: No recurrence was seen in 88% (193/219) cases, 12% (26/219) had recurrence (13 IC, 13 DCIS). FOXA1 and GATA-3 expression is maintained in 98% of ER(-) cases. We are reporting the initial results of biological marker expression in terms of recurrence. A high recurrence is seen with low GATA-3 expression (p=0.78) and low/absent PR (p=0.07).
Conclusions: A strong expression of FOXA 1, GATA-3, low Ki-67 index, and absent Her2 expression are characteristically seen in our ER(+) DCIS group, similar to previously described in IC. However, nearly all of the ER(-) cases expressed FOXA1 and GATA-3 which needs be evaluated further, as these transcription factors may offer new promising targets for therapy.

287 Delay to Formalin Fixation Effect on Estrogen and Progesterone Receptors in Breast Cancer: A Study of Three Different Clones

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Background: We have previously reported that delay to formalin fixation (DFF) is a factor that can negatively affect estrogen receptor (ER, clone 1D5) and progesterone receptor (PR, clone PgR636). Here, we further investigated the effect of DFF on ER (clones 6F11 and SP1) and PR (clones 16 and 1E2).
Design: Ten palpable invasive breast cancers were resected and underwent immediate gross evaluation. For each case, the procured tumor was divided into 8 parts and consecutively fixed after 0, 10, 30 minutes, 1, 2, 4, and 8 hours; one section was kept in saline and stored overnight at 4C°. Two tissue microarray (TMA) blocks were constructed. Three consecutive slides were stained with anti ER antibodies, clones 1D5, 6F11 and SP1 and 3 more consecutive slides were stained with anti PR antibodies, clones PgR636, 16 and 1E2 from DAKO, Leica and Ventana venders respectively. Q score was carried out recording both percentage of positive cells and staining intensity. Statistical analyses for pairwise comparison of groups were performed using the non-parametric sign test. A cumulative logist model was used to assess if the scores differed with the clone.
Results: All ten cases were invasive ductal carcinomas. There were 6 cases positive for ER and 4 cases positive for PR. For ER, mean Q score started to decline at 2 to 4 hour mark for clones 1D5 and 6F11 and 1 hr mark for SP1. Comparing each 2 clones, there was no statistically significant difference between them at each time mark, except between clones1D5 and SP1 at 8 hr mark. SP1 was superior to 1D5 at this hour mark (p=0.03). For PR, Q score started to decline at 1 hr mark for clones PgR636 and 16 and at 4 hr to 8 mark for 1E2. However, the difference was not statistically significant.
Conclusions: Regardless of antibodies clones, DFF has negative effect on hormonal receptors. Therefore, we recommend that specimens should not be delayed to formalin fixation for more than 1 hour and definitely not to be stored overnight.

288 Response to Trastuzumab-Based Neoadjuvant Therapy Does Not Correlate with Level of HER2 Amplification While Deletion of Chromosome 17 May Predict a Poor Pathologic Response

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Background: Trastuzumab-based therapy has relatively recently been used in the neoadjuvant setting for HER2+ breast cancer (BC). The reported rates of complete pathologic responses (cPR) in these patients range from 15% to 40%. The aim of this study was to identify pathologic variables predicting a cPR, and to assess the level of response with the level of *HER2* amplification.
Design: The pathology and medical records of women with HER2+ BC who underwent neoadjuvant trastuzumab-based therapy were reviewed between 2008-2009. Tumors were tested prior to neoadjuvant therapy for gene amplification by FISH using the PathVysion 2-color probe set. Deletion of chromosome 17 (del17) was defined as a mean

CEP17 copy number of ≤1.5 per cell. Additional pathologic data, including ER and PR status, were recorded. Estimated tumor volume reduction was based on the reported pre-neoadjuvant tumor size and the gross and microscopic estimation of residual tumor volume in the post-neoadjuvant specimen. T-test and correlation coefficient statistical analyses were performed using GraphPad software.

Results: Of the 29 cases included, 5 (17.24%) had cPRs. The mean estimated tumor volume reduction was 60% (range 0-100%), with 20.69% (6/29) showing <10% tumor volume reduction. The average HER2/CEP17 ratio was 5.40 (range 1.84-12.79). Neither the *HER2* copy number nor the HER2/CEP17 ratio correlated with percent tumor volume reduction (cc = 0.106 and -0.04, respectively). There was no significant difference in the mean *HER2* copy number or HER2/CEP17 ratio in the cPR group compared to the partial or non-responders. Eighty-percent (4/5) of the complete responders were ER-negative compared to 45.8% (11/24) of the remaining cases, but this difference did not reach statistical significance (p=0.33). However, tumors with del17 exhibited a poorer response than those without del17, with mean tumor reduction of 27.0% and 67.17%, respectively (p=0.0271).

Conclusions: Response to trastuzumab in the neoadjuvant setting is variable, and is likely related to complex molecular interactions rather than level of *HER2* amplification, while del17 may predict a relatively poor pathologic response. These data need to be validated on larger sample sizes for confirmation of these findings.

289 A Distinct Gene Expression Profile Is Associated with Aggressive Breast Carcinomas (BC) Showing Prominent Retraction Artifact (RA)

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Background: RA resulting in clear spaces around tumor cell nests is frequently seen in histologic material of BC and may present difficulty in its differentiation from lymphatic invasion (LVI). We have recently reported that the extent of RA in BC is a strong predictor of LVI and nodal metastasis and suggested that the characteristic clear spaces separating tumor cells from the stroma are not just a random artifactual phenomenon resulting from tissue fixation and processing, but rather, they are likely related to molecular alterations in BC resulting in altered tumor-stromal interactions which might play an important role in lymphatic tumor spread. Using microarray technology we examined whether extensive RA is associated with a specific gene expression signature in invasive human BC.

Design: Fifty and 21 samples of formalin-fixed paraffin-embedded (FFPE) invasive BC showing high (≥ 20%) and low (<20%) levels of RA, respectively, were macrodissected and RNA was isolated after DNase digestion. Sample quality was assessed by RLP13a ribosomal protein mRNA specific TaqMan quantitative real time PCR (q-RT-PCR) after reverse transcription. cDNA from the 71 invasive BC were subjected to whole genome gene expression analysis using Illumina's cDNA-mediated annealing, selection, extension and ligation (DASL) assay based bead arrays, which are specifically designed to allow expression analysis of more than 24,000 human genes from partially degraded RNA of FFPE tissues. Data analysis was carried out using the Significance Analysis of Microarrays (SAM) and Ingenuity Pathway Analysis software tools.

Results: Comparison of whole genome gene expression profiles of tumors with high and low levels of RA resulted in 126 differentially expressed genes at a significance level of p<0.001 and a fold change of >2. Functional analysis revealed that the differentially expressed genes mainly play roles in the control of cellular growth and proliferation, cellular assembly and organization, cellular development and cell death, with emphasis on the Wnt/β-catenin, FGF and TGF-β signaling pathways. Verification of whole genome DASL assay results are being carried out by TaqMan PCR and immunohistochemistry.

Conclusions: Our results suggest that aggressive BC showing extensive RA are assocuiated with a specific gene expression profile compared to tumors without this feature and support the hypothesis that extensive RA is a histologic reflection of important biologic changes resulting in altered tumor-stromal interactions and a propensity for lymphatic tumor spread.

290 Increased Erythropoietin Receptor (EpoR) Expression Is Associated with Distinct Gene Expression Profile in Human Breast Cancer (BC)

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Background: EpoR serves as a specific receptor for erythropoietin (Epo), a glycoprotein hormone that stimulates the proliferation and inhibits the apoptosis of erythroblasts in the bone marrow. Recombinant human Epo (rHuEpo) has revolutionized the treatment of anemia and is widely used in the management of cancer and therapy related anemia in patients with malignant diseases. Although rHuEpo is effective in correcting anemia and improving quality of life, recent clinical trials suggested that its use may be associated with earlier tumor recurrence/progression and decreased survival. Various human cancer cells were recently shown to express EpoR and in vitro studies suggested that Epo can stimulate signaling mechanisms and proliferation in some cancer cells providing a potential biologic basis for the observed adverse effects rHuEpo in cancer patients. However, currently the role for EpoR in cancer biology is unclear. We investigated whether increased EpoR expression in BC is associated with a distinct gene expression profile in human BC.

Design: Ninety-four macrodissected formalin-fixed paraffin-embedded invasive breast carcinomas were used for microarray analysis using Illumina's cDNA-mediated annealing, selection, extension and ligation (DASL) assay based bead arrays. Based on the expression of EpoR we selected 20 samples each showing the highest and lowest levels of EpoR expression for analysis. Significance Analysis of Microarrays (SAM) and Ingenuity Pathway Analysis software tools were applied for data analysis.

Results: Comparison of whole genome gene expression profiles of tumors with highest and lowest levels of EpoR expression yielded 584 differentially expressed genes at a significance level of p<0.001 and a fold change of >2. Genes (n=414) overexpressed in tumors with high EpoR expression included genes with functional roles in cell cycle regulation and proliferation and hematologic system development and function. Genes with decreased expression mainly play roles in the control of cellular development, movement and cell death. A significant correlation was found between high EpoR expression and estrogen receptor expression in BC (p=0.043).

Conclusions: Our results suggest that high EpoR expression in BC is associated with a distinct gene expression profile compared to tumors with low EpoR expression. Identification of such differentially expressed genes may help understand the biological role of EpoR in the tumor biology.

291 Histologic Spectrum of Magnetic Resonance Imaging (MRI) Detected Suspicious Lesions and Its Clinical Impact in Newly Diagnosed Breast Cancer Patients

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Background: Pathologic studies have shown that women thought to have localized breast cancer are at increased risk of having occult multifocal carcinoma which may not be detectable by mammography (MG) or ultra-sonography (USG). MRI is being increasingly used for preoperative local staging, especially in women with mammographically dense breast. We sought to determine the histologic spectrum of MRI detected suspicious lesions and its impact on management.

Design: There were 156 MRI guided biopsies performed at our institution from June 2007 to June 2009, 109 of which were in newly diagnosed breast cancer patients. All the slides were reviewed and pertinent pathologic and clinical parameters were recorded. Cases were broadly subclassified on the basis of pathologic diagnosis into three categories: benign, malignant [in-situ ductal (DCIS) or lobular (LCIS), invasive ductal (IDC) or lobular carcinoma (ILC)] and borderline [atypical duct hyperplasia (ADH), atypical lobular hyperplasia (ALH), flat epithelial atypia (FEA)]. Effort was made to determine whether MRI biopsy changed overall management.

Results: There were 109 MRI guided biopsies from 101 newly diagnosed breast cancer patients (76 IDC, 9 ILC, 14 DCIS, 1 LCIS, 1 carcinosarcoma). Out of 109 biopsies, 80 (73.4%) were benign (26 benign breast tissue, 13 fibroadenomas, 13 fibrocystic change, 9 sclerosing adenosis, 8 intraductal papillomas, 5 fat necrosis, 3 stromal fibrosis, 1 each radial scar, lymph node, stromal hyperplasia). 23/109 (21.1%) were carcinomas (15 DCIS, 7 IDC, 1 ILC); 9 of which were detected in the ipsilateral breast, 12 contralateral, 2 bilateral. There were 6 (5.5%) cases with borderline histology (4 ADH, 1 ALH and 1 FEA), 2 detected in the ipsilateral breast, 3 contralateral, 1 bilateral.

Conclusions: MRI detected significant (25.7%) additional occult disease which changed the course of management. These additional findings were found in bilateral breasts (2.8%), ipsilateral breast (10.1%) and slightly more frequently in contralateral breast (12.8%). But false positive (FP) rate was high (73.4%) and there is a need to reduce FP MRI detection. Correlation of histologic features with suspicious MRI findings may help fine tune radiologists interpretations to achieve this goal.

292 Mucinous Carcinoma of Breast: A Retrospective Review of 100 Cases over 9 Year Period with Emphasis on Axillary Staging

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Background: Pure mucinous carcinoma of breast (PMBC) has a better prognosis than mixed mucinous carcinoma (MMC). Although most studies claim this, reports of lymph node (LN) and distant metastases exist in PMBC. We evaluated patient demographics, pathological features and receptor status of mucinous breast carcinoma to address some of these questions.

Design: Two broad categories of a PMBC (n=45) with more than 90% mucinous component and a mixed type (n=55), with 50 to 90 % mucinous component were studied. PMBC was further subclassified as hypocellular/type A (n=37) and hypercellular/type B (n=8) based on the amount of mucin and cellularity. A retrospective analysis of these cases diagnosed at the three hospital centers between 2000 to 2009 was done. Clinicomorphological and limited prognostic features were compared. No patient follow up was done.

Results: Mean age at diagnosis in PMBC and MMC was 60 years (range 34-91), and 63 years (39-90) respectively. Mean tumor size in PMBC and MMC was 1.65 cm (range 0.2-3.5) and 2.5 cm (0.1-9.5) respectively. Mean age in type A and B was 75 and 55 years while mean tumor size was 1.4 and 1.9 cm respectively. Surprisingly we had only 1 case of PMBC with mucocele like lesion. Ductal carcinoma in situ (DCIS) was present in 58% of PMBC and 74% MMC. Well differentiated PMBC were 55.5% (64% type A and 13% B), moderately differentiated 33.3 % (27% type A and 63% B), while poorly differentiated were 4.4% (0% type A and 25% B). Sentinel lymph nodes (SLN) were positive in 18.5% PMBC (15% type A and 29% B), and 16% MMC. Non sentinel lymph node (NSLN+) was in 14% PMBC (10% type A and 25% B) and 39% MMC. All PMBC with LN(+) had micrometastasis while 40% MMC had macrometastasis. Lymphovascular invasion (LVI) was seen in 6% and 22% of PMBC and MMC respectively. 95% PMBC were ER(+), 84% PR(+) and 11% Her2(+) while, 91% MMC were ER (+), 87% PR(+) and 33% Her2(+).

Conclusions: In our study, SLN (+) was observed in a similar % of PMBC and MMC. Hence we emphasize a positive role of axillary staging in PMBC. Rather than tumor size, grade and nodal status should predict outcome and management in PMBC as most patients with LN (+) and high histological grade in our study, had a tumor size between 2 to 3 cm. Although limited by number of cases and statistical significance, type B was less favorable than A, comparing LN(+), histological grade, LVI and mean age. Her2(+) was higher in our study (11%) but this could relate to ploidy status of tumor, which requires further studies.

293 Immunohistochemistry (IHC) and In-Situ Hybridization (ISH) for HER2 on a Single Slide Is Feasible and Allows Direct Comparison at the Individual Cell Level

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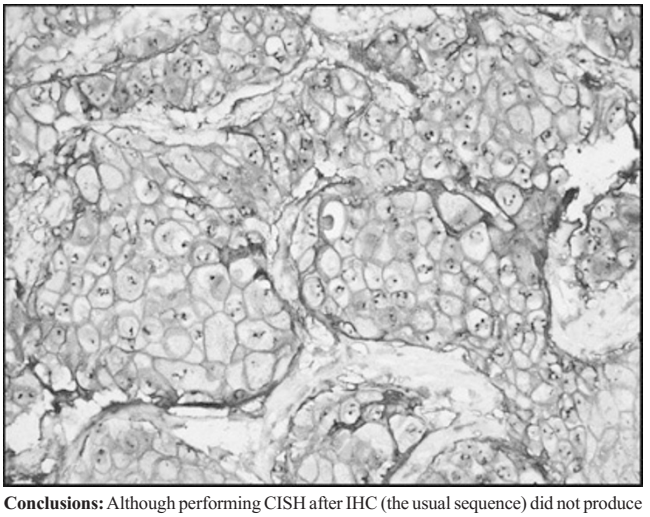
Background: Although either IHC or ISH may be originally used for HER2 testing in breast carcinoma (BC), repeat testing with a second method is often required for equivocal cases and validation. Given that chromogenic ISH (CISH) is similar to IHC in that conventional bright-field microscopy is used for its interpretation, we investigated the possibility of performing both HER2 IHC and CISH on a single section.

Design: Different sequential and combined protocols for the performance of HER2 CISH (Biocare) and IHC (Dako) on a single slide were evaluated on a BC tissue microarray (TMA) of 10 cases, a HER2-positive control case, and 9 archival BC cases, 3 of which were amplified.

Results: a) CISH after IHC: Multiple evaluations of the HER2-positive control case did not change the 3+ signal obtained when IHC was applied alone; however no CISH signals could be detected. b) IHC after CISH: This produced 100% IHC/CISH concordance in the TMA with three 3+/amplified cases and seven 0 or 1+/non-amplified cases. Evaluation of the HER2-positive control case with 45 min antibody incubation (vs. 30 min for IHC alone) produced a 3+/amplified profile identical to that seen in the individual IHC and CISH tests. c) Combined protocol (antigen retrieval; protease digestion; hybridization; antibody application; dual detection). Applying this on the HER2-positive control case produced the expected 3+/amplified profile (fig); however, there was a slight reduction in the HER2 staining intensity. This was more evident in archival cases where 2 of the 3 amplified cases showed a reduction from the 3+ signal seen by IHC alone to a 2+ signal; the remaining 9 cases had results identical to those in individual IHC and CISH tests.



Conclusions: Although performing CISH after IHC (the usual sequence) did not produce DNA signals, performing IHC after CISH or using a combined protocol to evaluate both on a single section is feasible and permits simultaneous "cell-by-cell" evaluation of HER2 expression and gene amplification status. The main issue is decreased HER2 expression, presumably due to membrane digestion; however, longer antibody incubation may correct this. Additional testing is being performed.

294 Histologic and Epigenetic Characterization of Pre- and Postmenopausal Breast Cancer from Women in Senegal, West Africa

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Background: African American women are at increased risk for premenopausal breast CA yet there is currently no cost effective way to screen for these cancers. Ultimately, we aim to develop serum biomarkers for early detection but molecular data are limited at present. As these cancers are relatively rare in the US, we chose to study breast CA among women in Senegal, West Africa. Our aims were to establish the frequency of premenopausal CA in this population, determine if the histology and risk factors of premenopausal breast CA in West Africans are similar to those in African Americans, and to begin to define the epigenetic profiles of such tumors.

Design: 522 consecutive women presenting to the Dakar Tumor Institute in Senegal, West Africa with a breast mass were enrolled and all women underwent a physical examination and medical history. Needle core biopsy and aspiration of the mass was preformed with subsequent histological evaluation. The epigenetic profile of these tumors was then assessed by examining the methylation status of 32 genes known to be involved in breast and other epithelial cancers.

Results: Of the 522 women enrolled, the presence of cancer was confirmed in 252. The average age of women with CA was 45.2 and 144 were premenopausal (57%). The mean gravidity was 5.7 and only 4% of women had any history of hormonal birth control use. Only 1% reported using tobacco while 2% reported using alcohol. 11 had personal history of breast CA (4%) and 10 had a family history of breast CA (4%). Tumor size, grade, nodal status, metastasis, and stage were determined. Of the 32 genes evaluated, there was a statistically significant difference in the methylation status between pre- and postmenopausal women in 8 of the candidate genes; 2 of the genes showed increased methylation in premenopausal women while the remaining 6 showed increased methylation in the postmenopausal women.

Conclusions: Breast CA, in this population, was most commonly premenopausal. Interestingly, the methylation status of tumors from pre- and postmenopausal women was found to be significantly different in 8 genes, suggesting the molecular biology of these tumors differs. Given the similarities in the genetic backgrounds of Western African and African American women, our results have important implications for the understanding of the molecular basis of premenopausal breast CA in these populations. In addition, these 8 genes are likely candidates to begin to develop biomarkers for the early detection of premenopausal breast CA.

295 Upgrade Rates of Lobular Neoplasia on Core Needle Biopsy: Should Atypical Lobular Hyperplasia Be Routinely Excised?

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Background: There is debate as to the best management strategy when lobular carcinoma in situ (LCIS) and/or atypical lobular hyperplasia (ALH) are identified on core needle biopsy (CNB). While some institutions consistently recommend excision due to the risk of upgrade to ductal carcinoma in situ (DCIS) or invasive carcinoma, others consider each on a case by case basis. Our institution has been routinely recommending excisional biopsy following diagnosis of ALH and LCIS on CNB since 2003. Consequently, we have a significant collection of cases with minimal selection bias allowing study of the frequency of upgrade rates in this group.

Design: All cases of lobular neoplasia (ALH and LCIS) diagnosed on CNB were identified from our pathology database since 2003, when surgical excision was first recommended. The imaging modality which prompted the biopsy and the pathologic findings on surgical follow-up were recorded. Upgrade rates to invasive carcinoma or DCIS for both ALH and LCIS were calculated. CNB cases with atypical ductal hyperplasia (ADH), DCIS, invasive carcinoma, or pleomorphic LCIS were excluded from analysis.

Results: 151 cases of lobular neoplasia (ALH, n=91 and LCIS, n=60) diagnosed from 2003-2009 on CNB were identified. Of these, 96 cases had lobular neoplasia without associated ADH or higher grade lesion on CNB, and 80 (83%) had available surgical follow-up. Overall, there was an upgrade rate of 10% to a more significant pathologic lesion (7.5% to DCIS and 2.5% to invasive lobular carcinoma). Of the 45 cases of pure ALH, there were no upgrades to DCIS. Only 1 ALH case upgraded to invasive lobular carcinoma (2.2%) and that lesion was associated with a mass on imaging. None of the cases of ALH biopsied for findings unrelated to a mass upgraded. LCIS on CNB (n=35) had a higher upgrade rate of 20%, with 17.1% upgrading to DCIS (n=6) and 2.8% upgrading to invasive lobular carcinoma (n=1). Interestingly, unlike the ALH case that upgraded, none of the upgraded LCIS lesions were associated with a mass on imaging.

Conclusions: In our experience, ALH diagnosed on CNB for non-mass related findings did not upgrade to DCIS or invasive carcinoma at surgical biopsy, suggesting routine excision may not be necessary in this setting. LCIS, however, had an upgrade rate similar to those reported for ADH, and excisional biopsy should continue to be offered to rule out an associated higher grade lesion. Finally, these findings suggest that ALH and LCIS on CNB carry inherently different risks of having associated invasive carcinoma.

296 Most Salivary Gland-Like Tumors of Breast Express Basal Type Immunohistochemical Markers

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Background: Salivary gland-like tumors represent approximately 2% of primary breast carcinomas. These special histologic subtypes are characteristically negative for ER, PR, and HER2 (triple-negative) and include adenoid cystic carcinoma, mucoepidermoid carcinoma, acinic cell carcinoma, and polymorphous low-grade adenocarcinoma . Approximately 75% of triple-negative breast carcinomas belong to the basal-like subtype by gene expression profiling. Immunohistochemical panels that include basal-like markers such as EGFR, CK5/6, p-cadherin, p63 and c-kit provide useful surrogates to gene expression arrays for classification of triple-negative breast cancers into the basal-like subtype. The purpose of this study was to explore the expression of these markers in salivary gland-like tumors of the breast.

Design: Excisional specimens from 10 untreated invasive triple-negative mammary carcinomas with salivary gland-like morphologies were evaluated for the immunohistochemical expression of EGFR, CK5/6, p-cadherin, p63 and c-kit using formalin-fixed, paraffin-embedded tissue and the L-SAB detection method.

Results: Based on morphology, 4 carcinomas were classified as adenoid cystic, 3 as mucoepidermoid, 2 as polymorphous low-grade and 1 as acinic cell. All of the adenoid cystic carcinomas, mucoepidermoid carcinomas and polymorphous low grade adenocarcinomas showed strong and diffuse expression of CK5/6, EGFR, p-cadherin, p63, and c-kit. The single case of acinic cell carcinoma expressed c-kit, but failed to express the other basal-like immunohistochemical markers.

Conclusions: Adenoid cystic, mucoepidermoid and polymorphous low-grade carcinomas of the breast express immunohistochemical markers that characterize the intrinsic basal-like subtype of breast cancer.

297 Stromal CD10 and SPARC Expression Predict Recurrence of Ductal Carcinoma In Situ

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Background: Ductal carcinoma in situ (DCIS) currently accounts for 20-30% of newly diagnosed breast cancers. The current classification of DCIS based on nuclear grade, architectural differentiation and the presence of necrosis does not adequately predict the likelihood of recurrence after breast conserving therapy. Therefore there is a critical need to identify novel predictors of DCIS progression. Recently, the stromal

proteins CD10 and SPARC (secreted protein acid rich in cysteine), identified in invasive breast carcinoma, have been correlated with decreased survival. There have been no studies addressing the clinical significance of stromal CD10 and SPARC expression in DCIS. Our study evaluated these proteins in DCIS and examined their association with clinicopathological variables and DCIS recurrence.
Design: Eighty five cases of DCIS were included in the study. All patients were treated with wide local excision alone. CD10 and SPARC expression in tumor stroma was assessed by standard immunoperoxidase method with anti-CD 10 (1:200 dilution, Burlingame, CA) and anti-SPARC (1:200 dilution, AbCam, Cambridge, MA)) antibodies. The staining was scored semi-quantitatively as negative (0; no staining), weak (1; either diffuse weak staining or strong staining in less than 30% of stromal cells) and strong (2; defined as strong staining of 30% of stromal cells). Statistical analysis was performed using the Fisher's exact test. Multivariable analysis was conducted utilizing Exact Logistic Regression software (SAS 9.1 and LogExact).
Results: The recurrence rate for our study population was 28%. There was a significant association between stromal CD10 expression and DCIS recurrence ($p<0.001$, Fisher's exact test). Recurrence was observed in 5% of patients with the CD10 stromal score 0 and in 63% of patients with the score 2. There was also a significant association between stromal SPARC expression and recurrence ($p<0.001$, Fisher's exact test). Among patients with the stromal SPARC score 0, only 7% had a recurrence whereas recurrence occurred in 50% of patients with the stromal score 2. There was no association between CD10 or SPARC expression and DCIS type, nuclear grade, estrogen receptor, progesterone receptor or HER2 status. In multivariate analysis stromal CD10 but not SPARC expression remained a strong predictor of DCIS recurrence.
Conclusions: Our results indicate that stromal CD10 and to a lesser extent SPARC are potentially novel biomarkers predicting DCIS recurrence.

298 Thyroid Transcription Factor (TTF-1) Expression in Breast Carcinomas

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Background: Immunostaining for thyroid transcription factor-1 (TTF-1) is frequently used to help assess the site of origin of metastatic carcinomas. TTF-1 is most frequently expressed by carcinomas of thyroid and lung origin. Furthermore, it has been assumed that expression of TTF-1 in a carcinoma excludes the possibility of a breast origin. We have recently encountered in our consultation practice three cases of invasive breast carcinoma (confirmed by clinical findings and other immunophenotypic features) that demonstrated unequivocal expression of TTF-1. Two of these cases had neuroendocrine features on H and E-stained sections (confirmed by chromogranin and synaptophysin immunostains); the third case had apocrine features on H and E-stained sections. However, the frequency with which TTF-1 expression is observed in breast carcinomas is unknown. The purpose of this study, therefore, was to evaluate the prevalence of TTF-1 staining in an unselected series of breast carcinomas.
Design: We performed immunostaining for TTF-1 (clone SPT24, Leica/Novacastra, 1:400 dilution) following heat induced epitope retrieval in 305 breast cancer cases submitted to a reference laboratory for routine estrogen receptor (ER), progesterone receptor (PR), and/or HER2 immunostaining. Cases were considered TTF-1 positive if they showed any nuclear staining for this marker.
Results: TTF-1 expression in tumor cell nuclei was seen in 4 of 305 of the breast cancers studied (1.3%). In 3 of the 4 cases, TTF-1 expression was weak and focal. In the fourth case, strong TTF-1 expression was seen in the nuclei of both invasive carcinoma and associated ductal carcinoma in situ. Of note, these 4 TTF-1 positive breast cancers varied with regard to their histologic type, histologic grade and ER, PR and HER2 status.
Conclusions: In this study, TTF-1 expression was seen in just over 1% of breast carcinomas and was most often focal and weak. There were no histologic features or ER/PR/HER2 profile that were particularly associated with the presence of TTF-1 staining in these cases. Thus, while diffuse, strong TTF-1 expression in a carcinoma likely excludes a breast origin, the presence of TTF-1 immunoreactivity cannot by itself be used to rule out a breast origin in a carcinoma of unknown primary site. These results emphasize once again the potential pitfall of relying on the results of a single immunostain to establish the site of origin of a carcinoma in a patient without a known primary site.

299 Microinvasive Lobular Carcinoma of Breast: Characterization of a Rare Entity Based on a Clinicopathological Profile of Fifteen Cases

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Background: Clinicopathological data on MicroInvasive (<0.1cm) Lobular Carcinoma (MILC) of Breast is scarce, and as such this pathological entity remains poorly characterized.
Design: All available clinical and pathological material on cases in which MILC was either suspected or diagnosed over an eighteen-year period (1991-2009) was reviewed. Only cases wherein the final diagnosis of MILC could be indisputably established by 2 observers, and confirmed via appropriate immunostains (cytokeratin (+), one or more myoepithelial markers (-), and E-cadherin (-)) were included. Cases wherein a larger invasive tumor was identified in a subsequent specimen were excluded.
Results: Fifteen (15) cases were confirmed to be MILC (in a search of 47,547 breast cases). Mean age of patients was 52 (range 41-65). Presentation included radiographic abnormality in 8 cases and mass in 3 (no such information in 4 cases). MILC was unilateral in all 15 cases (right: 7, left: 8). Mean number of microinvasive foci was 1.5 (range 1-5). All foci of MILC were intimately associated with lobular carcinoma in situ (LCIS). The associated LCIS was of classical type in all 15 cases, and nuclear grade of MILC cells and adjacent LCIS was low to intermediate. Synchronous pleomorphic LCIS existed in 1/15 (7%) case. Neither stromal reaction nor inflammatory infiltrate was present in any case and the only histological hint of MILC was enhanced stromal cellularity. Three cases had synchronous DCIS of solid, cribriform or micropapillary types. Each of 8 MILC cases, wherein results could be obtained, were found to be positive for estrogen receptor (ER) and progesterone receptor (PR) (MILC was not present in ER and PR stained sections of 7 cases). Her-2/neu was not overexpressed in each of 7 cases wherein results could be obtained (MILC was not present in HER-2/neu stained sections of 8 cases). The final surgical procedure was excisional biopsy (8 cases), ipsilateral mastectomy (5), and bilateral mastectomies (2). Eight of 15 cases had residual LCIS or atypical lobular hyperplasia. Ten of 15 cases had axillary lymph node dissection (with sentinel node biopsy in 8), with all lymph nodes being negative. All patients remain alive with no evidence of recurrence/metastases on a mean follow-up of 24 months (range 1-72 months).
Conclusions: MILC is a rare, histologically subtle, lesion associated with classical LCIS. MILC appears to be a low-morbidity disease with neither recurrences nor metastases observed (at least in the short term).

300 Cancer/Testis (CT) Antigens Are Preferentially Expressed in Hormone Receptor Negative Breast Cancers and May Offer Therapeutic Targets

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Background: CT antigens are proteins normally expressed only in germ cells and yet are aberrantly activated in a wide array of human cancers. This makes them attractive targets for cancer immunotherapy and phase III clinical trials are ongoing for melanoma and lung cancer. In breast cancer, we recently showed that 2 CT antigens, MAGE-A and NY-ESO-1, were preferentially expressed in estrogen receptor (ER) negative tumors. To further investigate the characteristics of CT expression in breast cancer, we analyzed the expression of 8 CT antigens, correlating results to ER, progesterone receptor (PR), and HER2 status of these tumors.
Design: A tissue microarray of 40 ER-positive and 39 ER-negative invasive breast cancers (71 ductal, 8 lobular) were analyzed. PR and HER2 status were recorded (34 PR-positive, 11 HER2-positive). The array was immunohistochemically stained for 8 CT antigens: MAGE-A, NY-ESO-1, GAGE, CT7, CT10, CT45, ACTL8, and NXF2. CT antigen expression was interpreted as positive when unequivocal nuclear staining was seen in any tumor cells.
Results: All CT antigens showed nuclear (CT10, CT45, ACTL8, NXF2) or mixed nuclear/cytoplasmic (MAGE-A, NY-ESO-1, GAGE, CT7) staining, with both diffuse and focal staining patterns. CT antigens showed preferential expression in hormone receptor-negative tumors; 85% of ER-negative tumors expressed at least 1 CT antigen, versus 20% of ER-positive tumors ($p<0.0001$). ER-negative tumors were also more likely to show simultaneous expression of >1 CT antigen than ER-positive tumors (49% *vs.* 5%, $p<0.0001$). HER2 status had less effect ($p=0.19$ for expression of any CT antigen), but HER2-positive tumors were more likely to express >1 CT antigen than HER2-negative tumors (73% *vs.* 20%, $p<0.001$). Among the 8 CT antigens, MAGE-A and CT7 were most frequently expressed, observed in 49% and 36% of ER-negative tumors, respectively, and 37% and 30% in the ER, PR and HER2 triple-negative subset.
Conclusions: ER-negative tumors showed significantly higher frequency of CT antigen expression, often expressing more than 1 CT antigen. HER2 status had less effect on CT expression. Our results indicate that CT-based cancer vaccine immunotherapy may potentially be useful in treating ER-negative breast cancers, including the triple-negative subset of carcinoma for which current treatment options are limited.

301 CXCL12/CXCR4 Transcripts Levels of Expression Are Linked to Distant Metastasis and DFS Outcome in Breast Carcinoma

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Background: Breast carcinoma is the main cause of cancer-related deaths in occidental women and is mainly due to distant metastatic disease. Recent works suggest an involvement of chemokine CXCL12 and CXCR4 in the metastatic process, in both stroma and tumor cells. We assessed the prognostic value of *CXCR4* and *CXCL12* transcripts in breast carcinoma and correlated these value with immunohistochemical staining and "classical" prognostic factors.
Design: In a series of 113 patients who had surgery between january 2001 and december 2003 with primary breast cancer, with a minimum follow-up 24 months and for which frozen materiel and paraffin embedded tumor was available we did the following analysis: Tissue micro-array Immunohistochemistry for antibodies: Estrogen Receptor, Progesterone receptor, HER2, Cytokeratins 5-6 and 14, CXCR4 (clone Ab2074 and clone MAB172). Real-time RT-PCR for CXCR4 short (CXCR4s) and long(CXCR4l) transcripts, CXCL12 transcripts alpha(CXCL12a), beta(CXCL12b) and gamma(CXCL12g).
Results: Among the patients included, with an average follow-up of 52 months, 12 developped distant metastasis. Patients with lymph nodes metastasis, high grade tumors and absence of hormonal receptors(HR) expression were more likely to develop distant metastasis. Immunohistochemical analysis with CXCR4 with either antibody was not correlated with distant metastasis or lymph node metastasis. There was poor correlation between different CXCR4 antibodies result (kappa 0.29) and no correlation between quantitative RT-PCR results and CXCR4 immunohistochemichal results. CXCR4s level was more elevated in patients who developped distant metastasis($p=0.05$) and seemed to have an adverse effect on DFS ($p=0.07$). CXCR4l showed lower level of expression than CXCR4s and also had an impact on DFS($p=0.06$). There was a slight correlation between grade and level of level of expression of both transcripts and grade ($p=0.08$). CXCL12a and CXCL12b levels were inversely linked to grade ($p=0.03$) and HR expression ($p=0.03$). There was no relationship between CXCR12 transcripts and distant metastasis or lymph node metastasis.
Conclusions: Our work confirms a potential involvement of the CXCR4/CXC12 signalling in metastatic process. For the first time, the impact of alternative transcript

is assessed and suggests new physiopathological mechanisms. It also shows that CXCR4 immunohistochemical staining is not the best way to evaluate the role of these molecules.

302 Comparative Assessment of Immunohistochemistry (IHC), Fluorescence In Situ Hybridization (FISH), and Silver-Enhanced In Situ Hybridization (SISH) for Evaluation of HER2 Gene Status in Breast Carcinoma

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Background: HER2 gene amplification occurs in 15% to 25% of breast cancers and has implications for treatment and prognosis. The established and approved method for HER2 gene status is IHC in combination with FISH techniques. Recently, new direct evaluation technique, silver enhanced in situ hybridization (SISH) was developed. This study was performed to evaluate SISH technique for clinical use.

Design: We studied 729 cases of excised breast carcinoma specimen by IHC (anti-HER2 Mouse Monoclonal Antibody, NeoMarkers, Fremont, CA USA), FISH (PathVysion® HER-2 DNA Probe Kit, Abbott/Vysis Abbott Park. ILL USA), and SISH (INFORM® HER2 SISH DNA probe, Ventana, Tucson, AZ USA) using tissue microarray (TMA; AccuMaxTM array, ISU Abxis, Seoul, Korea) and evaluated accordance of two techniques. Interpretation SISH, IHC and FISH was performed according to Interpretation Guide INFROMTMHER2 (Roche) and ASCO/CAP guideline, respectively.

Results: Her2 gene status could be assessed by IHC, FISH and SISH in 504 cases. The IHC results show 498 (score 0), 13 (score1), 29 (score2), and 99 cases (score3). The results of FISH show 369 (negative), 0 (equivocal), and 135 cases (positive). The results of SISH show 363 (negative), 6 (equivocal), and 135 cases (positive). 7 cases among 369 cases with Her2 negativity in FISH showed Her2 gene amplification in SISH. The accordance rate of SISH and FISH is 98.4% (kappa value 0.96).

Conclusions: SISH technique is effective modality comparable with FISH to evaluate HER2 gene amplification in breast carcinoma.

303 Infiltrating Pleomorphic Lobular Carcinoma: High Oncotype DX Score Augments Its "Aggressive" Reputation

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Background: Emerging data indicate that the Pleomorphic Variant of Infiltrating Lobular Carcinoma (PV-ILC) has more aggressive potential, and significantly worse recurrence-free survival than the Classical Variant of Infiltrating Lobular Carcinoma (CV-ILC). PV-ILC and CV-ILC share E-cadherin negativity and a common molecular pathway; however, the biological profile of PV-ILC remains relatively unexplored.

Design: Estrogen receptor (ER)-positive breast carcinoma cases with concurrently obtained Oncotype DX® Recurrence Score (ODX-RS) [a 21-gene commercially available molecular test (Genomic Health, Inc, Redwood City, CA)] were categorized for morphological type (ductal versus lobular, confirmed with E-cadherin immunostain) and conventional Nottingham grade(1-3). ODX-RS and 10-year recurrence rate (RR), both as reported by ODX, from patients with PV-ILC were compared to those with CV-ILC and infiltrating duct carcinoma (IDC) of all 3 grades.

Results: The results of 153 ER-positive cases with concurrent ODX-RS and 10-year RR are tabulated below for PV-ILC and CV-ILC, well differentiated-IDC (WD-IDC), moderately differentiated-IDC (MD-IDC), and poorly differentiated-IDC (PD-IDC).

	n	RS	10-year RR	p value
PV-ILC	09	**23.6**	15%	-
CV-ILC	23	**14.8**	9.9%	0.004*
WD-IDC	29	**15.5**	10.1%	0.002*
MD-IDC	54	**16.4**	10.8%	0.007*
PD-IDC	38	**27.6**	17.8%	0.42

n: number of cases, RS: ODX-RS, RR: Recurrence Risk, *statistically significant

Conclusions: ODX-RS even in ER-positive PV-ILC are significantly higher than in CV-ILC, WD-IDC and MD-IDC; and are statistically similar to those obtained in PD-IDC. Our data further augment the mounting evidence that PV-ILC is an "aggressive" tumor.

304 *TMEPAI* Gene Amplification in Triple Negative Breast Cancers

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Background: Transmembrane, prostate androgen induced protein(TMEPAI), an androgen-induced, cell-cycle-regulated protein that is encoded by the *TMEPAI* gene on chromosome 20, has demonstrated overexpression in many cancers including breast. *TMEPAI* may play a significant role in tumorigenesis by preventing apoptosis and promoting invasiveness or migration of breast cancer cells. Only a few human breast cancers have been examined for *TMEPAI*, and this study was undertaken to look at the incidence of *TMEPAI* gene amplification in human breast cancer, particularly in triple negative cancers (ER-, PR-, HER2-).

Design: Fresh frozen tissues from 97 cases of breast cancer, including 85 invasive ductal carcinomas, 11 invasive lobular carcinomas and 1 mixed ductal and lobular carcinoma, were assayed by Agilent (Santa Clara, CA, USA) human genome CGH microarray 105A. Analysis was performed using Agilent (Santa Clara, CA, USA) genomic workbench 5.0 software. The locus chr20:55,656,858-55,719,947 for *TMEPAI* was visualized in each of the 97 breast cancer cases and degree of expression was noted as loss (-0.2 to <-0.7), normal (< 0.2 to >-0.2), gain (0.2 to < 0.5), or high copy gain (> 0.5). The *TMEPAI* findings were then correlated with histologic grade (Nottingham score) and breast prognostic markers as measured by immunohistochemistry and *in situ* hybridization, including ER, PR and HER2.

Results: 47/97 breast cancers (48.5%), including 45/85(53%) invasive ductal carcinomas and 2/11(18%) invasive lobular carcinomas, showed either gain (26/97, 26.8%) or high copy gain (21/97, 21.6%) of *TMEPAI*. The majority of the tumors with copy number gain were histologic grade 3 (34/47, 72.3%). Of the breast cancers with gains or high copy number gain, 25 were ER-negative (53.2%), 31 PR-negative (66%), and 36 HER2-negative (76.6%). In all, 18 of 31 triple negative cancers showed gene amplification of *TMEPAI* in our group of breast cancer cases (58.1%).

TMEPAI gene status and breast cancer characteristics

	Ductal	Lobular	Grade 3	ER-	PR-	HER2-	Triple-
TMEPAI +	45	2	34	25	31	36	18
TMEPAI nl	40	9	24	18	23	39	13

Number of cases listed

Conclusions: *TMEPAI* gene is commonly amplified in breast cancers (47/97, 48.5%), particularly in ductal carcinomas, including the majority of triple negative tumors (18/31, 58.1%). In addition, most of the breast tumors showing *TMEPAI* gene amplification were grade 3 tumors (34/47, 72.3%). Amplification of *TMEPAI* may be one of the factors that increase the aggressiveness of triple negative breast carcinomas.

305 Association of PIK3CA Mutations in Exon 9 with Luminal Immunophenotype Breast Carcinoma

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Background: The phosphatidylinositol 3-kinase subunit PIK3CA is frequently mutated in human cancers. The most frequent reported mutations are G1624A (E542K) and G1633A (E545K) in exon 9 (helical domain), and A3140G (H1047R) in exon 20 (kinase domain). The aim of our study was to investigate the relationship between the presence of PIK3CA mutations and tumor characteristics in a series of Luminal A and B, and HER2+ immunophenotypes of breast carcinoma (BC).

Design: In this control-case study, we analyzed PIK3CA mutations in 373 human BC samples: 146 Luminal A (ER and PR >50% positive nuclei; and p53 <20% or Ki67 <20%), 47 Luminal B (ER and/or PR <50%, and p53 >20% or Ki67 >20%), and 180 HER2+ (>30% 3+ or amplified by FISH). We used allelic discrimination based on real-time chemistry TaqMan MGB probes in ABI Prism 7500 Sequence Detection System (Applied Biosystems). Direct DNA sequencing of exons 9 and 20 in ABI Prism 310 confirmed all positive samples. Immunohistochemical (IHC) staining was performed in whole sections for HER2, ER and PgR (cut-off 10%), Ki-67, p53 (cut-off 20%) and Bcl2 (cut-off 50%). Chi-square and Fisher's exact tests were used for statistical analysis of qualitative variables and T-Student test or U. Mann-Whitney test for quantitative variables.

Results: We identified PIK3CA mutations in 25.2% (94/373) tumors: 30.6% in Luminal subtypes and 19.4% in HER2+ (p=0.013; OR:1.824 (1.129-2.947)). Stratification of Luminal tumors into A and B versus HER2+ showed mutations in 31.5%, 27.7% and 19.4%, respectively (p=0.041). Mutations in exon 9 (G1624A and G1633A) were more frequently detected in Luminal than in HER2+ tumors (p=0.057 and p=0.006, respectively) but no differences were found for those in exon 20 (A3140G) (p=ns). Mutated phenotype correlated negatively with the proliferation marker Ki-67 (p= 0.007) (with a median of 10% (range 5-20%) for mutated, and 17% (range 8-27%) for wild-type cases); and positively with Bcl2 expression (p=0.014, OR:1.860 (1.131-3.059)). Interestingly, the presence of mutations was associated with bilateral tumors (p=0.021, OR=0.208 (0.048-0.901)).

Conclusions: Our findings show that PIK3CA mutations in exon 9 (G1624A and G1633A), are associated with good prognostic factors, such as Luminal A and B immunophenotypes BC, low proliferation index and Bcl2 overexpression. Supported by Grant FIS 06/1495, and FCVI-HGUA (2009 PI-C/03).

306 Histopathology of the Male Breast: Eleven Years Experience of the Vanderbilt Breast Consultation Service

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Background: Male invasive breast cancer (IBC) is rare with an incidence of 1% among all breast cancers. Atypical ductal hyperplasia (ADH) in men is extremely rare. Where as gynecomastia, a benign enlargement of the male breast as a result of proliferation of the glandular component, is common, being temporarily present in 30-50% of healthy men. Diagnoses of ductal carcinoma in situ (DCIS) as well as ADH are indications for total mastectomy in men. Given the prevalence of gynecomastia and the rarity of ADH in males, strict criteria for diagnosis of ADH must be applied.

Design: The authors report the variety of male breast pathology seen in consultation by the Vanderbilt Breast Consultation Service over an approximately 11 year period from December 1997 to September 2009.

Results: Cases from 79 males averaging 52 years of age (range 13-99 yrs) were received in consultation. Eight IBC (6 intermediate grade no special type, 1 low grade no special type, and 1 special type pure invasive cribriform carcinoma), 14 cases of ductal carcinoma in situ (9 low grade, 4 intermediate grade, and 1 high grade with Paget's disease), 42 cases of ADH (3 involving papillomas), 13 cases of gynecomastia without atypical hyperplasia, and 2 benign stromal lesions (myofibroblastoma and cellular leiomyoma) were diagnosed. All but the stromal lesions and 3 of the invasive cancers showed at least focal background gynecomastia. IBC and DCIS each showed a non-overlapping bimodal age distribution (average ages 33 and 71 years, respectively) where as the diagnosis ADH showed a uniform distribution with age (13-82 yrs).

Conclusions: The most frequent male breast lesion seen in consultation was ADH arising in a background of gynecomastia. The same histologic criteria used for diagnosis of ADH in the female breast should be rigidly applied to avoid unnecessary mastectomy. The uniform age distribution for ADH in contrast to the bimodal distribution of carcinomas suggests ADH is an important marker of risk which differs with age. Male breast cancer

is rare even in a consultation setting (less than 1 case per year). The histopathology of these lesions is identical to their female counterparts.

307 Continued Observation of the Natural History of Low-Grade Ductal Carcinoma In Situ in Women Treated by Biopsy Only Reaffirms Proclivity for Local Recurrence in the Original Site over More Than 30 Years Long-Term Follow-Up

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Background: Opportunities to study the natural history of ductal carcinoma in situ (DCIS) are rare. A few studies of incompletely excised lesions in the premammographic era now recognized as DCIS have provided critical insights into its proclivity for local recurrence in the original site. At the time the biopsies in the current study were originally examined, small DCIS was not diagnosed and, by default, these women were treated by biopsy only.

Design: The authors report the latest results from a follow-up study, which was published originally in 1982, now of 45 women with DCIS who were treated by biopsy only. These women were from a large, prospectively identified, completely characterized cohort.

Results: Sixteen of 45 women developed invasive breast carcinoma (IBC), all in the same breast and quadrant from which their DCIS biopsy was taken. Twelve IBCs were diagnosed within 10 years of the DCIS biopsy, 1 was diagnosed within 12 years of the DCIS biopsy, and the remaining 3 IBCs were diagnosed over 23-42 years. Seven of these women, including 1 woman who developed IBC 29 years after her low grade DCIS biopsy, developed distant metastasis, which resulted in death 7 years after the diagnosis of IBC.

Conclusions: The natural history of low-grade DCIS can extend greater than 4 decades, with IBC developing at the same site as the previous DCIS in the majority of women. This natural history differs markedly from that of patients with high-grade DCIS and from the outcome of patients with any completely delimited DCIS excised to negative margins.

308 Microarray Based Determination of ER, PR and HER2 Receptor Status Compared to Local IHC/FISH Assessment

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Background: The level of estrogen receptor (ER), progesterone receptor (PR) and HER2 expression is predictive for prognosis and/or treatment response in breast cancer patients. However, differences in fixation and IHC and subjective interpretation can substantially affect the accuracy and reproducibility of the results. The recently developed TargetPrint test measures the mRNA expression level of ER, PR and HER2 and provides an objective and standardized alternative to IHC. This study compares results from the microarray-based (MA) TargetPrint with IHC and FISH generated by local standard procedures.

Design: Material and methods: Prospective tumor samples were collected for 53 patients diagnosed with breast cancer stage I to III between 12/08 and 08/09. The mRNA level of ER, PR and HER2 and the MammaPrint risk analysis were assessed in a central laboratory (Agendia BV, Amsterdam) in fresh tumor samples submitted from 3 European and 1 Japanese hospital: The results of the IHC/FISH assessments performed according to the local standards at the hospitals were compared to the quantitative gene expression readouts.

Results: Of the 53 samples 4 samples were ineligible for gene expression profiling. In the remaining 49 samples, 1 had insufficient RNA quality and 48 (98%) could successfully be hybridized. Median age of these patients was 60.4 years. Among the 48 patients, 19 (40%) were classified as good prognosis, whereas 29 (60%) were classified as poor prognosis. Comparison of IHC and MA showed a concordance of 92% for ER; 83% for PR and 92% for HER2. Submitted from Bamberg, 5 of 11 patients assessed by IHC and MA had initially a discordant PR result. Re-analysis of all patients using a new antibody for the PR staining confirmed the microarray results in 4 patients.

Conclusions: Microarray based readout of ER, PR and HER2 status using TargetPrint is highly comparable to local IHC and FISH analysis on retrospectively and prospectively analyzed samples in various hospitals. Moreover, TargetPrint confirmed a suspected PR staining problem in one hospital suggesting that TargetPrint microarray readouts for hormone and HER2 receptor status in addition to standard IHC can improve the molecular characterization of breast cancer tissue.

309 Intratumoral Heterogeneity of HER-2/neu Gene Amplification in Breast Cancer

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Background: Genetic heterogeneity of HER-2 gene amplification has been reported in variable frequencies in breast cancers.

Design: To determine the frequency of intratumoral heterogeneity of HER-2 gene amplification and find the characteristics of tumor with it, we evaluated 3 cores of tissue microarray from different areas of each tumor by dual color fluorescence in situ hybridization in 50 invasive breast carcinomas (IBCs) with HER-2 gene amplification on whole tissue sections and 2+ or 3+ HER-2 protein overexpresssion. HER-2 gene amplification was scored by the American Society of Clinical Oncology/College of American Pathologist (ASCO/CAP) criteria. HER-2 genetic heterogeneity was defined as the existence of tumor cells with a ratio higher than 2.2 in 5% to 50% of tumor cells according to CAP guideline.

Results: Of the 50 IBCs, 24 cases (48%) showed high grade (HER-2/CEP17 ratio = or > 4), 15 (30%) showed low grade (ratio >2.2 to <4.0) and four cases (8%) showed high or low grade amplification in three cores. Seven cases (14%) showed discordant results for HER-2 amplification. Of two cases with high grade amplification in a core, one case showed not amplified pattern in other cores, and the other case showed not amplified pattern or equivocal HER-2 gene amplification in other cores. Of five cases with low grade amplification in at least one core, two case revealed equivocal HER-2 gene amplification and the other two cases revealed chromosome 17 polysomy in other cores. HER-2 genetic heterogeneity was found in six (12%) cases, all of which showed discordant results for HER-2 gene amplification.

Conclusions: Assessment of HER-2 gene amplification seems to be not significantly affected by intratumoral heterogeneity in most IBCs. However, HER-2 genetic heterogeneity is present in a subset of IBCs, especially in some cases with low grade amplification and rare cases with high grade amplification. Further studies will be needed to determine the clinical significance of HER-2 genetic heterogeneity in breast cancers.

310 Piwil2-Like (PL2L) Proteins Are a Potential Biomarker for Breast Cancer Progression

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Background: The significance of piwil2 as tumor biomarker has been controversial. Recently, we have generated two monoclonal antibodies (mAbs) Kao1 and Kao2, which recognize piwil2 (Kao1) and piwil2/PL2L proteins (Kao2), respectively. We hypothesize that PL2L proteins rather than piwil2 are expressed in various developmental stages of tumor such as breast cancers.

Design: 400 breast tumor specimens including benign proliferation [ductal hyperplasia (DH) and metaplasia (DM), n=9], precancer [atypical ductal hyperplasia (ADH, n=5), and ductal carcinoma in situ/ductal cancerization (DCIS, n=6), and malignant cancer [invasive ductal carcinoma (IDC) and metastatic cancers (Met-ca), n=379] were prepared as tissue microarrays (TMAs) and were immunohistochemically analyzed for piwil2 and PL2L proteins expression. Because piwil2 and NF-kB are implicated for tumor stem cell (TSC) development, some TMAs were doubly stained with mAb Kao2 and NF-kB.

Results: Among 400 breast cancer specimens, only was <1% of the TMA cores stained within an apoptotic focus by Kao1 mAb, whereas >95% of the TMA cores was stained by Kao2 mAb with individual variations, suggesting that PL2L proteins rather than piwil2 were dominantly expressed in breast cancers. While almost all $Kao2^+$ cells were co-stained in cytoplasm by mAb to NF-kB, not all $NF\text{-}kB^+$ cells were co-stained by Kao2 mAb. Interestingly, some $Kao2^+NF\text{-}kB^+$ cells were also stained in nucleus (< 1% to 35% of $Kao2^+NF\text{-}kB^+$ cells). The nuclear Kao2+NF-kB+ cells appeared to be associated with tumor progression, because these cells were rarely detected in the benign proliferation lesions (DH and DM) and precancerous lesions (ADH) except for DCIS, about 50% of which was detected with nuclear $Kao2^+NF\text{-}kB^+$ cells, especially in the ductal cancerization areas. These results suggest strongly that nuclear $Kao2^+NF\text{-}kB^+$ cells might signify histological and clinical progression of breast cancers.

Conclusions: PL2L proteins rather than piwil2 are dominantly expressed in breast cancers, which have the potential to be used as a breast cancer biomarker for early detection. The nuclear $Kao2^+FN\text{-}kB^+$ cells might be significant for prediction of breast tumor progression and prognosis.

311 The Oncotype DX Assay (ODX) May Not Be Required for All Estrogen Receptor (ER) Positive Breast Cancers

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Background: The ODX is a 21-gene RT-PCR based commercial assay that is being increasingly used in the management of ER positive (+), lymph node negative (-) breast cancers. The assay provides prognostic and predictive information in the form of a recurrence risk score (RS) that separates patients into low, intermediate, or high risk. This study is designed to determine if histologic and conventional immunophenotypic features of breast cancer are able to predict the ODX RS.

Design: Morphologic features of 153 invasive breast cancers from 3 different institutions excised between 2006 and 2009 were compared to the ODX RS. Features examined included Modified Bloom-Richardson score (MBR), ER, progesterone receptor (PR), Her2/neu status and Ki67 proliferation index. ER/PR were analyzed using the Allred scoring system. Allred percentile scores of 0-4 were considered negative for this study. Ki67 was scored based on percent positivity: <10%= low, 11-20% = intermediate and >20% = high. Expression and amplification were evaluated for Her2.

Results: 66 (43%) of the cancers had a low RS, 66 (43%) an intermediate RS, and 21 (14%) had a high RS. A general trend of increasing ODX RS with higher MBR, higher Ki67 proliferative rate, loss of PR, and overexpression of Her2 was noted. 23 (15%) cases were MBR 1, ER+, PR+, Her2-, and low Ki67. 22 of these had a low ODX RS while 1 had an intermediate RS. Analysis of the 21 cases with a high ODX RS revealed the following: 6 cases had an equivocal or unknown Her 2 status and were excluded. Of the remaining cases 9/15 (60%) had a high Ki67 score, 4/15 (27%) were ER-, 8/15 (53%) were PR- (this included all 4 of the ER- tumors), and 6/15 (40%) overexpressed Her2. In fact, 14/15 (93%) had one or more of the following poor prognostic features: high Ki67 score, ER-, PR-, Her2+. In addition, all cases with MBR of 3, high Ki67 and negative PR and all cases with a high Ki67 and positive Her2 had a high ODX RS. Only 1 case with a high ODX RS was ER+, PR+, and Her2-. This tumor had MBR 2 with an intermediate Ki-67.

Conclusions: Carcinomas with a constellation of good prognostic indicators (MBR 1, low Ki-67, PR+, Her2-) are likely to have a low ODX RS while those with a combination

of poor prognostic indicators (MBR 3, high Ki67, PR-, Her2+) are likely to have a high RS. Better understanding of the conventional prognostic markers may obviate the need for ODX testing in a subset of breast carcinomas.

312 Biomarker Expression Can Identify Biologically Distinct Categories of Invasive Breast Cancer

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Background: Tumor size, stage, lymph node status and histologic grade are significant prognostic factors in breast cancer. We sought to investigate the usefulness of a panel of biomarkers by correlating them with clinicopathologic parameters and outcome.

Design: Tissue microarrays from 1158 patients with stage I and II invasive breast cancer were constructed and immunohistochemical staining for ER, PR, HER2, P53, Ki-67, CK5/6, CK14, CK17, EGFR, Bcl-2, COX-2 and P27 were performed.

Results: The positive rates for ER, PR, HER2, P53, CK5/6, CK14, CK17, EGFR and COX-2 were 66%, 53%, 10%, 57%, 10%, 5%, 9%, 8% and 84%, respectively. The mean percentage of Bcl-2 was 70% and that of P27 was 62%. Ki-67 positivity was low in 61%, intermediate in 26% and high in 14%. Table1 summarizes the correlation between the biomarkers and clinicopathologic parameters. Many biomarkers were associated with each other. In univariate analysis, tumor size and stage were predictive of disease-free survival, while ER, CK14, EGFR, tumor size and stage were predictive of overall survival.

Table1.Correlation between markers and clinicopathologic parameters shown by P-value

	Size	Stage	Nuclear grade	Metastasis
ER	<0.0001	0.0001	<0.0001	0.043
PR	0.002	0.004	<0.0001	0.464
HER2	0.170	0.160	<0.0001	0.218
CK 5/6	<0.0001	0.0001	<0.0001	0.022
CK 14	<0.0001	0.004	<0.0001	0.039
CK 17	0.0003	0.003	0.014	0.060
EGFR	0.0008	0.002	<0.0001	0.217
P53	0.056	0.176	0.0002	0.415
COX-2	0.0003	0.588	<0.0001	0.819
Bcl-2	<0.0001	0.0001	<0.0001	0.081
P27	0.0003	0.020	<0.0001	0.047

Conclusions: Our large cohort of patients with long term follow-up confirms that evaluation of known biomarkers is necessary for all breast cancers. Current clinical practice might benefit from the addition of basal cytokeratins, EGFR, P53 and COX-2 to current panels.

313 Florid Lobular Carcinoma In-Situ (FLCIS): Molecular Profiling and Comparison to Classic LCIS (CLCIS) and Pleomorphic LCIS (PLCIS)

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Background: Lobular carcinoma in-situ (LCIS) shows a range of morphologic variants including CLCIS, PLCIS and apocrine (apo) PLCIS. Classification & management for these subtypes remain problematic. Molecular profiling of LCIS subtypes may reveal relationships of these subtypes within the lobular neoplastic pathway. FLCIS is a distinct subtype characterized by classic type LCIS cells with direct involvement/marked expansion of ducts, central necrosis, calcifications, and increased frequency of concurrent invasive carcinoma (invca). Although the morphologic and immunohistochemical features of FLCIS have been described, its molecular profile has not yet been reported.

Design: Genomic alterations were evaluated on 20 cases of FLCIS using array-based comparative genomic hybridization (aCGH) with BAC arrays. Biomarker expression was examined by immunostaining for E-cadherin (E-cad), ER and cyclin D1. The genetic characteristics of FLCIS were compared to those of 20 CLCIS and 21 PLCIS (including 8 apo) from our previously published data performed on a similar aCGH platform.

Results: Similar to CLCIS and PLCIS, FLCIS was characterized by 1q gain (80%) and 16q loss (100%). Other recurrent genomic alterations observed in FLCIS included loss of 11q (50%), 17p (40%) and 8p (25%), and amplification including the *cyclin D1* gene at 11q13.3 (25%). All 20 cases were E-cad negative and 19 (95%) were strongly positive for ER. Cyclin D1 expression was higher in cases with *cyclin D1* gene amplification. Compared to CLCIS, FLCIS displayed significantly more fraction genome loss (4.6% v. 2.2%, p=0.007), more chromosomal breakpoints (11.6 v. 5.9, p=0.002) and higher numbers of amplifications (2.10 v. 0.15, p=0.03). FLCIS cases also showed significantly more chromosome breakage than PLCIS (p=0.04), and similar genetic complexity as seen in apo PLCIS.

Conclusions: FLCIS shares the cytologic features, E-cad loss and the lobular genetic signature of 1q gain and 16q loss found in CLCIS. However, these lesions demonstrate more genomic alterations than CLCIS. Our data support the conclusion that FLCIS is a genetically more advanced lesion than CLCIS. This may explain the greater frequency of concurrent invca in FLCIS than in CLCIS. These alternations also lend support for clinically managing this variant of LCIS more like DCIS than CLCIS.

314 Clinicopathologic Characteristics of Carcinomas That Develop after a Biopsy Containing Columnar Cell Lesions

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Background: Columnar cell lesions (CCL) of the breast are frequently detected mammographically because they often contain calcification. Because of their frequent association with atypical ductal hyperplasia, lobular neoplasia, and tubular carcinoma, they have been suggested as a precursor lesion for low grade carcinomas. However, in long term follow-up studies, CCL are associated with only a slight increase in later breast cancer development (Boulos et al. Cancer 113:2415-21, 2008). If CCLs are precursor lesions, we would expect subsequent cancers to develop in the same site as the biopsy. Moreover, we would expect the subsequent carcinomas to be of low grade. Our goal was to review the clinical and pathologic features of carcinomas that develop after a diagnosis of CCL to try to establish these lesions as precursors to invasive carcinoma.

Design: From the Nashville Breast Cohort, 1261 biopsies were identified that contained CCL; these formed the basis of a nested case control study. CCL were defined using the criteria of Schnitt and Vincent-Salomon (Adv Anat Pathol 10:113-24, 2003). In follow up of an average of 17 years, 77 women developed invasive carcinoma, compared to 152 controls who did not develop invasive carcinoma. The clinical and pathologic features of the cancers that developed form the basis of this study.

Results: CCL were more likely to be associated with atypical ductal hyperplasia, (ADH) at the time of original biopsy, although the presence of CCL did not increase the risk over that of ADH alone. The average age at diagnosis of invasive carcinoma was 63 years. The majority of invasive carcinomas that developed were of no special type, and were of intermediate combined histologic grade. Moreover, the carcinomas that developed were as likely to occur in the contralateral breast as in the breast that was originally diagnosed with CCL.

Conclusions: There is a favored association of CCL with atypical ductal hyperplasia. Subsequent carcinoma development is uncommon, and may occur in either breast. The development of intermediate grade carcinomas of no special type, argues against CCL being an obligate precursor lesion for invasive carcinomas.

315 Calretinin Expression in Poorly Differentiated Breast Carcinomas with an Emphasis on the Basal-Like Subtype

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Background: Basal-like subtype of breast cancer was identified by gene expression profiling. These tumors express basal cytokeratins and epidermal growth factor receptor (EGFR), but lack estrogen receptor (ER), progesterone receptor (PR) and HER2. Because this subtype has a poor prognosis, there has been a consistent effort to refine its morphological and immunophenotypic features. Calretinin is one of the calcium binding protein. It is expressed in a variety of normal tissues and several malignancies. Our goal was to investigate the expression pattern and diagnostic utility of calretinin in the basal-like subtype of breast carcinoma.

Design: Paraffin embedded microarray tissue from 248 consecutive poorly differentiated invasive ductal carcinomas was analyzed for expression of calretinin(mouse monoclonal antibody, clone Z11-E3, Zymed). On the basis of expression of ER, PR, HER2, cytokeratin-5/6 and EGFR the cases were stratified into luminal (ER+/PR+), HER2 positive (ER-/PR-/HER2+), and basal-like (ER-/PR-/HER2-/CK5/6+ and / or EGFR+) subtypes. The extent and intensity of calretinin expression were scored semiquantitatively using a combined score on a scale of 0-3+.

Results: The cases were stratified into 71 luminal, 71 HER2, and 94 basal-like. Positive calretinin staining was characterized by the presence of both nuclear and cytoplasmic reactivity. Of the total 248 poorly differentiated carcinomas calretinin expression was seen in 90 (36%) cases. Calretinin positivity was found in a significant proportion of the basal-like cancers (55/94,59%). In this group, the majority of positive cases (48 of 55, 87%) demonstrated strong (2+, 3+) calretinin positivity. In contrast, luminal and HER2 subtypes showed calretinin expression in only 24% & 25% of cases, respectively (p<0.001). There was a significant positive association of expression of calretinin with cytokeratin 5/6 and EGFR (p<0.001).

Expression of calretinin in poorly differentiated invasive ductal carcinoma

Tumor subtype	Frequency	Calretinin positive	Strong calretinin positive (2+-3+)
Luminal	71(29%)	17/71(24%)	10/71(14%)
Her-2 neu	71(29%)	18/71(25%)	11/71(16%)
Basal	94(39%)	55/94(59%)*	48/94(51%)*

*p < 0.001 vs luminal and Her-2 neu+

Conclusions: This study is the first to demonstrate strong calretinin expression in the basal-like subtype of invasive breast carcinomas. Based on our findings, calretinin may be useful as an additional biomarker to support the diagnosis of the basal-like subtype.

316 Correlation of Oncotype Dx Recurrence Score and Receptor Assessment with Histologic Parameters and ER/PR/Her-2 Status

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Background: Oncotype Dx (ON) is a commercially available 21 gene RT-PCR assay that provides a recurrence score (RS) for patients with ER + node negative breast carcinoma. This is included in the NCCN guidelines (2009) for a subset of patients with small tumors (0.6 to 1 cm) but with unfavorable histology. These reports have now also started providing ER, PR and Her 2 scores even though these are routinely reported by pathologists based on immunohistochemistry (IHC) or FISH tests.

Design: Our aim was to compare the ER, PR, and Her-2 results from ON reports (on excision specimens), with those using IHC or FISH from patients at our hospital (n=48). We also compared the RS with several histologic parameters including the Nottingham histologic score/grade, and isolated tumor cells (ITC) in lymph nodes. ON reports (from Genomic Health) provide scores with positive/negative cut-off at 6.5 units for ER, 5.5 units for PR and ≥ 11.5 units for Her 2. IHC was performed on optimally fixed breast core biopsies. Her-2 was evaluated utilizing the CAP/ASCO guidelines. ER and PR assessed on IHC sections were reported as negative (0) or % positive without any cut off value.

Results: All tumors were ER + by ON and by IHC. The score for ON ranged from 7.3 to 12.4 (median 9.6) and for IHC from 40 to 100% (median 95%). There was concordance for PR results in 43/46 cases (93.5%). The 3 tumors with discordant PR results had minimally + scores by ON or IHC. All cases were negative for Her-2 by ON, IHC and

FISH. 30/48 (62.5%) cases had a low, 14/48 (29.16%) an intermediate and 4/48 (8.33%) a high RS. 8/11(72.8%) grade 1 tumors had a low risk RS. None of them had a high risk RS (p=0.009). Ten tumors were histologic grade 3 and their RS were variable. Micropapillary histology and multifocality did not correlate with RS. 39/41 (95%) tumors measuring ≤ 2 cm had low RSs (p=<0.0001). Larger tumors had a variable RS. Ten cases had either ITC or a micrometastasis and 9/10 (90%) had a low RS.

Conclusions: This study shows a complete concordance between ER, as well as between Her-2 status by RT-PCR (ON) and IHC. It affirms that receptor status confirmed on core biopsies is reliably comparable to that performed on excision specimens. Histologic grade 1 tumors predict for absence of a high RS. An invasive carcinoma ≤ 2 cm is predictive of a low RS and presence of ITC in lymph nodes does not predict for a high RS.

317 *MYC* Gene Amplification in Breast Cancer Metastases Relative to Matched Primary Tumors

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Background: In breast cancer, the *MYC* proto-oncogene transcription factor is implicated in cell growth, transformation, cell cycle control and angiogenesis. Roles in endocrine resistance and tumor progression have been proposed. *MYC* gene amplification in primary tumors correlates with aggressive phenotypes (such as high grade and proliferation rates) and poor outcomes. Whether *MYC* amplification develops in metastases of unamplified tumors has not been systematically addressed.

Design: Fifteen rapid autopsies (post mortem intervals less than 4 hours) were performed on patients who died of metastatic breast carcinoma. Single patient tissue microarrays were constructed from paraffin tissue blocks from the patients' archived primary breast tumors and multiple metastases harvested at autopsy. ER/PR/HER2 and CK5/6 expression was assessed, and the cases characterized as Luminal, Basal-like (BLC) or HER2 based on published criteria. FISH was performed on the 15 TMA slides using a centromere (CEP) 8 probe and a *MYC* gene probe. The ratio of *MYC* to CEP8 signals was calculated for each of 145 primary tumor spots, and 778 spots derived from 180 different metastases. *MYC* duplication was defined as a MYC: CEP8 ratio of 1.3-2.0, and *MYC* amplification was defined as a *MYC:* CEP8 ratio of > 2.0. Results from the primary tumors were correlated with those of the patient's matched metastases.

Results: Six of 15 cases (4 Luminal, 1 HER2, 1 BLC) showed no evidence of *MYC* amplification in the primary or any metastatic site. Three cases (2 Luminal, 1 BLC) demonstrated *MYC* duplication and 2 cases (1 Luminal, 1BLC) demonstrated *MYC* amplification in the primary tumor and all metastases. Interestingly, 4 cases (27% of overall cases; 2 BLC, 1HER-2, 1 Luminal) demonstrated *MYC* amplification in metastases (2.62 to 6.16-fold higher copy number) compared to the matched primary. We were surprised to find relatively little heterogeneity in *MYC* amplification between different metastases from the same patient.

Conclusions: Amplification of *MYC* is a frequent event in breast cancer and may occur relatively late in tumorigenesis, present more often in metastatic disease than the corresponding primary. These observations underscore the importance of *MYC* in breast cancer progression/metastasis, as well as its relevance as a potential therapeutic target in otherwise incurable metastatic disease.

318 Lipocalin-2/NGAL Expression Is Associated with Clinical Outcome in Invasive Mammary Carcinoma

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Background: Lipocalin-2 functions as a transporter of small lipophilic ligands and has been shown to induce the epithelial to mesenchymal transition and tumor invasion in experimental breast cancer models.

Design: Formalin-fixed, paraffin-embedded tissue sections from 146 cases of invasive mammary carcinoma (102 ductal carcinomas (IDC) and 44 lobular carcinomas (ILC) were immunostained by automated methods (Ventana Medical Systems Inc., Tucson, AZ) using rat monoclonal lipocalin-2/NGAL (R&D Systems, Minneapolis, MN). Cytoplasmic immunoreactivity was semiquantitatively scored based on staining intensity and distribution and the results were correlated with morphologic and prognostic variables.

Results: Weak cytoplasmic staining of benign breast epithelium was identified in 100% of cases. Intense cytoplasmic lipocalin-2 overexpression was observed in 78/146 (53%) of invasive carcinomas. Lipocalin-2 overexpression correlated with PR positive status overall [63% PR positive versus 41% PR negative, p=0.012], within the IDC subgroup [63% PR positive versus 35% PR negative, p=0.009], and within the ER negative subgroup [80% PR positive versus 32% PR negative, p=0.007]; disease recurrence overall [69% recurrent versus 48% non-recurrent, p=0.021], within the IDC subgroup [70% recurrent versus 42% non-recurrent, p=0.011], and within the ER negative subgroup [72% recurrent versus 29% non-recurrent, p=0.004]; while the subgroup of positive tumors exhibiting intense diffuse lipocalin-2 overexpression 54/146 (37%) correlated with shortened survival within the ER negative subgroup [42% expired versus 15% alive, p=0.046] and the converse within the ER positive subgroup [32% expired versus 55% alive, p=0.027]. There was no correlation between lipocalin-2 expression and HER2 status (FISH). On multivariate analysis, advanced stage, lipocalin-2 overexpression, ER negative status, metastatic disease at diagnosis and positive node status were independent predictors of disease recurrence; while advanced stage, metastatic disease at diagnosis and positive node status were independent predictors of shortened survival.

Conclusions: Lipocalin-2 overexpression in invasive mammary carcinoma is significantly associated with hormone receptor status and independently predicts disease recurrence after primary treatment. Further study of this biomarker in breast cancer patients appears warranted.

319 Demographic, Clinical and Radiological Factors in Ductal Carcinoma In Situ of the Breast in Different Ethnic Groups: An Analysis of 75 Cases

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Background: Demographic data associated with invasive ductal carcinoma (IDC) have been extensively studied; however, data is relatively limited for ductal carcinoma in situ (DCIS). DCIS accounts for approximately 25% of all newly diagnosed breast cancers in women and due to extensive mammographic screening, its incidence has risen. The purpose of this study was to examine demographic, clinical and variables in DCIS that may predict risks of recurrence and outcome in different ethnic groups.

Design: We carried out a retrospective study of 75 cases of DCIS diagnosed in 1998-1999 and collected clinical data for 36 months or more of subsequent follow-up. The age at diagnosis, race, clinical and radiological presentation, DCIS grade & recurrence were recorded.

Results: African American (AA) patients comprised 25/75 (33%) of the cases, with 61 yrs average age at diagnosis. Average age of non-AA cases was 55 yrs. at diagnosis, being were significantly younger [t- test, p = 0.012]. DCIS was detected as a cluster of micro-calcifications on screening mammography in 17/24 (71%) AA cases compared to 40/50 (80%) in non-AA. In contrast, radiological masses were more frequently detected among AA cases 7/24 (29%) as compared to non-AA cases 10/50 (20%) [p=0.276, NS]. The pathologic specimens were graded as low (LG)-, intermediate (IG)-, and high-grade (HG) DCIS. Among the AA cases, 9/25 (36%) were LG, 7/25 (28%) IG, and 9/25 (36%) were HG DCIS. Among non-AA cases, 14/50 (28%) were LG, 14/50 (28%) IG and 22/50 (44%) HG DCIS [p=0.341, NS]. Recurrence was detected in 7/75 (9%) within 27 to 104 months of the therapeutic procedure. Both the recurrences among AA cases were HG DCIS (100%), while only 1/5 of the recurrences among non-AA was HG (20%). The cases had been followed for an average of 109 months from the time of diagnosis (46 to 131 months).

Conclusions: Our data suggests that DCIS occurred at an older age among AA cases. There is a trend towards a higher grade of recurrence among the AA cases. However, there is no statistically significant difference in the radiographic and pathologic features of DCIS between AA and non-AA cases. Further exploration of factors responsible for these trends within a larger cohort would help guide the optimal management of women who are at an increased risk of recurrence.

320 Hematolymphoid Neoplasms of the Breast: A Clinicopathologic Study of 58 Cases

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Background: Nonepithelial neoplasms of the breast, including hematologic neoplasms, are rare. There are few recent case series defining the clinicopathologic spectrum of breast hematolymphoid neoplasms according to World Health Organization (WHO) 2008 criteria. We report our experience with the diagnosis of breast hematologic neoplasms and discuss their clinical associations and implications.

Design: 58 hematolymphoid neoplasms of the breast were identified in our databases from 1999-2009 and reviewed along with clinical and laboratory data. Primary breast hematolymphoid neoplasms were designated if the infiltrate was closely associated with breast tissue and there was no prior diagnosis of lymphoma/leukemia or concurrent extramammary disease.

Results: In 58 cases, patients ranged from 3 to 82-years-old (median 63 years) and were predominantly female (96%). Of the total cases, 49 (84%) were B-cell non-Hodgkin lymphoma (NHL), including 16 follicular lymphomas (FL), 12 diffuse large B-cell lymphomas (DLBL), 11 marginal zone lymphomas (MZL), 4 mantle cell lymphomas, 4 CLL/SLL, 1 B-lymphoblastic lymphoma, and 1 unclassified lymphoma. The remaining cases were 3 anaplastic large cell lymphomas (ALCL), 2 Hodgkin lymphomas (HL), 2 granulocytic sarcomas, 1 follicular dendritic cell sarcoma (FDCS), and 1 extramedullary plasmacytoma. Primary neoplasms comprised 31% of total cases, including DLBL (7), MZL (5), FL (1), ALCL (1), and FDCS (1). Additionally, 7% of cases were associated with prior or subsequent breast carcinoma in the same or opposite breast, and 16% were associated with a prior or subsequent diagnosis of NHL, HL, or both. In 4 of 58 cases (2 primary), patients had a connective tissue disease and developed NHL (3 DLBL, 1 MZL), and 2 of 3 patients with ALCL (ALK-) had a prior diagnosis of sarcoidosis.

Conclusions: Secondary hematolymphoid neoplasms of the breast predominated (69%), with the majority being small mature B-cell neoplasms. Of the primary breast hematologic neoplasms, the majority were DLBL, followed by MZL. 11 of 58 cases were associated with prior or subsequent breast carcinoma or lymphoma, raising the possibility of therapy causing the secondary neoplasms. One breast carcinoma was diagnosed 6 years after lumpectomy and radiation for primary MZL, highlighting the importance of histologic evaluation of new lesions. DLBL was the most common hematologic breast neoplam in patients with connective tissue disorders (7% of total). Two of three cases of ALCL (ALK-) occurred in patients with sarcoidosis, an association that may be further investigated.

321 Incidental Minimal Atypical Lobular Hyperplasia on Core Needle Biopsy: Correlation with Findings on Follow-Up Excision

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Background: Atypical lobular hyperplasia (ALH) is usually an incidental finding in breast core biopsies (CB) that are performed for microcalcifications or mammographic densities. ALH is largely considered to be a risk factor for invasive carcinoma rather than a direct carcinoma precursor. There are relatively few series that focus on the management of ALH identified on CB, the results of which are controversial. Moreover, ALH is not separated from LCIS in these studies. We review our experience with incidental minimal ALH diagnosed on CB over the past 10 years, and correlate with follow-up excision results.

Design: We evaluated all cases of ALH diagnosed on CB from 1999-2009, only including cases that 1) had 3 or fewer foci of ALH (minimal ALH) on CB not associated with discordant calcifications or a mass 2) had follow-up excision; and 3) did not contain another lesion that by itself would require excision (such as atypical ductal hyperplasia (ADH) or intraductal papilloma). Cases in which the clinical and radiological impressions suggested that a mass lesion had been missed on CB, or in which the calcifications seen on mammography did not match those seen in the CB, were excluded. Therefore the excisional biopsies in these cases were performed because of the diagnosis of ALH.
Results: We identified 39 cases of incidental minimal ALH during this time period. Thirty-four CB (87%) were performed for microcalcifications, with the remaining 5 (13%) done for a mass or other radiographic abnormality. On follow-up excision, 23 cases (59%) showed residual ALH and 14 cases (36%) were benign. Only 2 cases had atypical lesions other than ALH (5%): 1 case had LCIS, and 1 case had focal mild ADH away from the biopsy site.
Conclusions: In our retrospective series, none of the cases had a lesion on excision that would have required further treatment, suggesting that these patients could have been managed more conservatively. We propose that minimal incidental ALH (limited to 3 or fewer foci) on CB does not require re-excision, if there is close radiological correlation, clinical observation, and follow-up.

322 Recurrence Score of 21-Gene Assay Is Correlated with a Panel of Immunopathological Factors of Breast Cancer

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Background: We have previously reported that higher recurrence score (RS) of 21-gene assay (Oncotype DX) is strongly associated with progesterone receptor (PR) negative breast cancer. Here with a larger case number, we sought to investigate the relationship between RS and a panel of immunopathological features of breast carcinomas.
Design: We identified 77 infiltrating carcinomas (70 IDC and 7 ILC) that had 21-gene assay tested from our departmental file and analyzed the relationship between the RS and clinicopathological factors. Immunohistochemical analyses were performed for ER, PR, HER2, EGFR, CK5/6. ER and PR were recorded as Allred scores. HER2 was scored as positive if >30% of tumor cells showed 3+ membrane staining. EGFR was designated as positive if any tumor cells showed 1+ positive stain. Any strong cytoplasmic stain was considered as positive for CK5/6. The definitions for each molecular subtype were based on the expression of ER, HER2, EGFR and CK5/6.
Results: Among the 77 cases, 46 had low RS (0-17), 26 had intermediate RS (18-30), and 5 had high RS (>31). PR expression was inversely associated with RS, with the mean Allred scores =7.72, 6.35 and 2.00 in low, intermediate and high RS group, respectively ($p<0.0001$). RS was also significantly associated with tubal formation, mitosis and luminal B subtype. Based on our data, a regression equation = 17.489 + 2.071 (tubal formation) + 2.926 (mitosis) – 2.408 (PR) – 1.061 (HER2) + 7.051 (luminal A) + 29.172 (luminal B) predicted RS with an R^2 of 0.65, which classified 59/77 cases into the same risk group as the assay RS. For the discrepancy between calculated RS and assay RS, 6 cases that had higher calculated RS compared to assay RS were tumors that were HER2 positive, high grade, or low PR expression; 12 cases that had lower calculated RS were tumors with high ER and PR expression, and low to intermediate histologic grades. The expression levels of PR and HER2 were also very important in the discrepancy between histologic grade and assay RS.
Conclusions: PR negativity, HER2 over-expression and high histologic grade are strongly correlated with a higher RS. More studies are needed to further investigate these relationships.

323 Degree of Intratumoral EGFR and Cytokeratin 5/6 Expression Is Significantly Associated with Nodal and Distant Metastases in Patients with Basal-Like Triple-Negative Breast Carcinoma

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Background: Triple-negative (TN) breast carcinoma, characterized by estrogen receptor, progesterone receptor, and Her2 negativity, is a group of aggressive tumors that can be further classified into two subtypes: basal-like, defined as cytokeratin (CK) 5/6 positivity and/or EGFR positivity by immunohistochemistry, and non-basal-like. Developing a novel treatment strategy to treat basal-like TN tumors is crucial for improving the prognosis of patients with these tumors.
Design: Clinical characteristics and tumor profiles were analyzed in 103 patients with TN tumors. Of these patients, 34 had distant metastatic disease, 34 had axillary nodal metastasis only, and 35 were nodal negative. The expression of basal cytokeratins (CK5/6, CK14, and CK17), EGFR, PTEN, and VEGF were compared between the distant metastatic, nodal metastatic, and nodal negative groups.
Results: There was no significant difference in the frequency of expression of these biomarkers between the 3 subgroups. However, among EGFR positive or CK5/6 positive cases (basal-like tumors), the combined nodal and distant metastatic group was found to have a significantly higher intratumoral expression of both CK5/6 ($p<0.05$) and EGFR ($p<0.05$) compared with the nodal negative group. There was no significant difference in the intratumoral expression of CK14, CK17, PTEN, and VEGF between subgroups of the basal-like tumors. In addition, the tumor size was significantly larger in the distant metastatic group as compared to the nodal metastatic or nodal negative groups ($p<0.05$, $p<0.001$ respectively), and the distant metastatic group had a significantly higher percentage of positive axillary lymph nodes in the axillary dissection as compared to the nodal metastatic group ($p<0.05$).
Conclusions: Our results indicate that basal-like TN breast tumors with nodal and distant metastases are significantly associated with a higher intratumoral expression of both EGFR and CK5/6 compared to those in the nodal negative group. Degree of intratumoral EGFR and CK5/6 expression may play a role in the development of nodal or distant metastases in patients with basal-like TN tumors and may be predictive of metastatic disease. Furthermore, EGFR targeted therapy may be potentially useful in the treatment of basal-like TN breast cancer. In addition, larger tumor size and a higher percentage of positive axillary lymph nodes are more likely to be associated with distant metastases in TN breast cancer.

324 Automated Bright-Field Dual Color, Dual Hapten HER2 In Situ Hybridization (DISH) Assay: An Alternative Method for Evaluation of HER2 Gene Amplification in Breast Cancer

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Background: HER2 positivity characterizes approximately 20% of invasive breast cancers and is evidenced by protein overexpression and/or gene amplification. Trastuzumab, an anti-HER2 monoclonal antibody, inhibits HER2-related tumor proliferation thus increasing patient survival. FISH is currently the standard method for assessment of HER2 gene amplification. Recently developed chromogenic detection systems such as silver ISH offer complete automation, fast turnaround times and bright-field microscopy, presenting an attractive alternative to FISH. We evaluated the performance of a new fully automated dual color HER2 in situ hybridization assay (DISH, Ventana) in comparison with FISH.
Design: FFPE blocks from 68 invasive breast cancers were tested by FISH (PathVysion, Vysis/Abbott) and DISH (Ventana). DISH was performed on Ventana BenchMark® series instrument. The assay allows visualization of the HER2 and chromosome 17 (chr 17) probes on the same slide. The HER2 probe is detected via silver deposition and the chr 17 probe is detected with a fast red and naphthol phosphate reaction. The cases were independently scored by 2 pathologists. The signals were enumerated in at least 20 tumor nuclei at 40X, and scored using the 3-tiered system recommended by ASCO/CAP (HER2/chr 17 > 2.2: amplified; 1.8 - 2.2: equivocal; < 1.8: non-amplified), and the 2-tiered system detailed in the DISH assay manual (HER2/chr 17 ≥ 2: amplified; < 2: non-amplified).
Results: The 68 cases consisted of 61 primary tumors and 7 metastases, 85% were resection specimens. Sixty-four (94%) were successfully analyzed. The overall concordance between DISH and FISH was 98% with the 3-tiered scoring system and 97% with the 2-tiered system. Repeat rate for DISH was similar to FISH (15% vs. 13%). Lack of interpretable signals on repeat analysis was recorded as failure and was slightly higher for DISH compared to FISH (6% vs. 2%). Some difficulty in DISH scoring was noted in cases with polysomy 17 and low-level amplification due to overlapping HER2 and chr 17 signals. Overall, DISH interpretation was straightforward and reproducible (96% interobserver concordance).
Conclusions: We found good concordance between the DISH and FISH in unequivocally non-amplified and amplified tumors. The DISH assay can be considered a reliable technique for the assessment of HER2 status.

325 ER+PR- Breast Carcinomas Are More Frequently Associated with Older Patients, Larger Tumors and HER2 Positive Tumors

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Background: Previous studies showed that ER+/PR- tumors are frequently seen in elderly women and in grade 2 ductal carcinomas. We also showed that these tumors are more frequently associated with bone metastasis and higher recurrence scores of 21-gene assay. In current study, we sought to investigate the clinicopathologic difference between ER+/PR-(PN) tumors and ER+/PR+(PP), ER-/PR-(NN), and ER-/PR+ (NP) tumors from a large data base.
Design: Nine hundred twenty consecutive cases of breast cancer between 1997 and 2008 were identified from our departmental file. Clinicopathological information such as patients' age, tumor type, tumor size, histologic grade, nuclear grade, lymph node status, and expression of ER, PR and HER2 were recorded. Statistical analyses were performed to compare among these 4 groups of tumors.
Results: Among the 920 cases, 546 (59.3%) were invasive ductal and ductal carcinoma in situ (IDC/DCIS), 190 (20.7%) were pure IDC, 118 (12.8%) were pure DCIS, and 66 (7.2%) were invasive lobular carcinoma (ILC). 165 cases (18.0%) were NN, 13 (1.4%) were NP, 101 (10.9%) were PN, and 641 (69.7%) were PP. The mean ages were 57.3, 53.1, 64.1, and 59.9 for NN, NP, PN, and PP, respectively, with PN being the oldest group and a p-value of 0.0004. The mean tumor sizes were 2.79cm, 1.85cm, 2.13cm, and 1.99cm for NN, NP, PN and PP, respectively, with a p-value of 0.0002. The most common histologic grades were grade 3 for NN (85%) and NP (55%), and grade 2 for PN (39%) and PP (40%), with a p-value less than 0.0001. The most common nuclear grades were grade 3 for NN (85%) and NP (70%); and grade 2 for PN (61%) and PP (57%), with a p-value less than 0.0001. HER2 over-expression was seen in 21%, 0%, 15%, and 9% in NN, NP, PN and PP, respectively, with a p-value of 0.0143. Lymphovascular invasion was seen in 26%, 8%, 15% and 17% in NN, NP, PN, and PP, respectively, with a p-value of 0.0351. Lymph node metastasis were similar among all 4 groups of tumors (p=0.2321).
Conclusions: PN tumors consist of 11% of all tumors, and are different from PP, NN, and NP tumors. They are more likely seen in older patients, with larger tumor and higher frequency of being HER2 positive compare to PP tumors.

326 Triple Negative Breast Cancers: Outcome Correlation with Immunohistochemical Expression of Basal Markers

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Background: Triple negative (TN) breast cancers are defined by the absence of estrogen receptor (ER), progesterone receptor (PR) and c-erbB2 expression. Oncologic

management options for this group of aggressive tumors are limited. Our data on 653 TN cancers disclosed 84% to be basal-like based on an immunohistochemical tripanel of CK14, EGFR and 34βE12. In this study, we evaluate their disease free and overall survivals (DFS, OS) in correlation with immunohistochemical expression of basal markers (CK5/6, CK14, CK17, 34βE12), SMA, p63, CD117 and EGFR.
Design: The study cohort comprised 653 TN breast cancers previously interrogated using antibodies to CK5/6, CK14, CK17, 34βE12, p63, CD117 and EGFR applied to sections cut from tissue microarray (TMA) blocks, using the streptavidin-biotin method. Follow-up was obtained from casenotes. DFS and OS were defined as time from diagnosis to recurrence or death respectively, and correlated with protein immunohistochemical expression. A p value <0.05 defined statistical significance.
Results: Median age was 52 years. Majority (82%) were Chinese, 8% Malay, 5% Indian, and 5% of other ethnic origins. Tumor size ranged from 0.9 to 20 cm (mean 2.9 cm, median 2.5 cm). Infiltrative ductal carcinoma was the commonest subtype (92%). Histologic grade 3 tumors predominated (77%). Node positivity occurred in 40%. CK5/6, CK14, CK17, 34βE12, SMA, CD117, p63 and EGFR were expressed in 6%, 48%, 50%, 70%, 25%, 45%, 22% and 30% of TN breast cancers respectively. Follow-up ranged from 1 to 185 months (mean 84, median 88 months). Recurrences occurred in 20% and deaths in 24% of women. Recurrences constituted local disease recrudescence (20%), distant metastases (49%), and both (6%), while contralateral breast cancer occurred in 25%. Both DFS and OS were statistically associated with CK5/6, CK17, 34βE12, p63, CD117 protein reactivity in an adverse manner, while SMA appeared to implicate a more favorable prognosis.
Conclusions: TN breast cancers in our study were usually high grade T2 tumors, with basal-like phenotype in up to 84%. Protein expression of CK5/6, CK17, 34βE12, p63 and CD117 is accompanied by more frequent recurrences and deaths, indicating that basal-like features as reflected by these markers are prognostic, and may support a need for their routine evaluation in TN cases. The presence of SMA, a marker of myoepithelial differentiation, in implicating a better OS, and the possibility of CD117 as a therapeutic target, require further study.

327 Prospective Multicenter Study of a Novel Fully Automated Molecular Test for Identification of Metastatic Carcinoma in Axillary Sentinel Lymph Nodes in Breast Cancer – The US OSNA Breast Cancer Sentinel Lymph Node Study Group

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Background: Molecular analysis of lymph nodes is useful for accurate, objective and rapid evaluation of the entire node for metastatic carcinoma. We assessed the performance of a novel, fully automated molecular test, One-step nucleic acid amplification (OSNA) Breast Cancer System, for detection of metastatic carcinoma in axillary sentinel lymph nodes (SLN) in a large prospective trial in comparison to detailed histopathologic(HP) examination.
Design: SLNs from early stage breast cancer patients were sliced (1-mm) using a proprietary 5-blade lymph node cutter. Alternate slices were analyzed using the OSNA system or deatiled HP examination. The OSNA system detects CK19 mRNA and was calibrated to detect tumor deposits >0.2 mm. OSNA results were classified as negative (<250 copies/µl); + as micro (≥250 copies/µl); or ++ as macrometastases (≥5000 copies/µl). The performance of OSNA system was compared with that of HP by calculating overall agreement, sensitivity and specificity using exact binomial 95% confidence intervals.
Results: We studied 1044 SLNs from 496 patients enrolled in 4 academic and 7 community centers. HP examination revealed metastatic carcinoma in 138 SLNs (13%), including 95 macrometastases (69%) and 43 micrometastases (31%). The OSNA system detected 86 (62%) of those macro and 21 (15%) of those micrometastases. The OSNA system also detected 9 macro (6%) and 29 micrometastases (20%) that were negative by HP. The discordance in the results between the two methods was proven to be due to tissue allocation bias and technical factors. Overall, the OSNA system detected metastases in 145 SLNs (14%). Analysis of up to three SLNs was completed in 45 minutes. The overall agreement, sensitivity, and specificity of the OSNA system on a SLN basis when compared to HP was 95.8%, 82.7%, and 97.7%, respectively (after discordant sample analysis (DCA)). After DCA, all OSNA ++ results were deemed to reflect nodal disease. The OSNA system performed well in academic and community hospitals.
Conclusions: 1) The performance of the OSNA Breast Cancer System was comparable with that of conventional HP examination in detecting metastatic carcinoma in SLNs. 2) The assay has features such as full automation, easy to learn and use, semi-quantitative results (+ = micromets and ++ = macromets) and rapid analysis, which makes it suitable for intraoperative and/or permanent comprehensive evaluation of SLNs in any pathology laboratory for routine patient care.

328 Expression Profile of Matrix Metalloproteinases in Primary and Metastatic Breast Cancer

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Background: Matrix metalloproteinases (MMP) are proteolytic enzymes degrading extracellular matrix components and playing crucial role in cancer development, progression and metastasis. The objective of this study was to determine mRNA and protein expression levels of collagenases MMP1, 2, and 9 in invasive ductal carcinoma (IDC) of breast, and their correlation with developing regional and distant brain metastases.
Design: mRNA levels of MMP were quantified by real-time RT-PCR in 12 frozen specimens including 4 primary IDC, 4 brain metastases and 4 normal breasts (NB), as well as in 6 human breast cancer cells lines, including IDC, and 1 subline, established from nude mice IDC brain metastasis. Tissue microarrays included 22 primary IDC, 17 brain metastasis (including 5 matched with primary), 5 lymph node metastasis (all matched), and 5 NB tissues. MMP protein expression levels were examined by immunostaining, quantified using automated imaging system (ACIS) and analyzed as integrated optical density (IOD) of brown staining per 10 mkm2.
Results: Human breast cancer cell lines demonstrated elevated mRNA levels of all studied MMP. Frozen tissues showed only MMP9 mRNA overexpression with trends of higher mRNA levels in IDC compared to NB or brain metastases. Immunostaining of primary and metastatic breast cancers also showed significant differential expression for MMP9. In brain metastases protein expression was present in 4/17 cases for MMP1, 1/17 for MMP2 and 15/17 cases for MMP9 (Anova p-values 0.91, 0.78 and 0.031 respectively). MMP9 IOD was higher in lymph node metastases vs IDC (p=0.024), and brain metastases vs primary IDC (p=0.01). It trended higher in IDC vs NB (p=0.056). We found strong positive correlation between MMP9 IOD in lymph node and brain metastases (p<0.05).
Conclusions: Enhanced MMP9 expression in metastatic breast cancer vs primary and normal breast tissue was found at both mRNA and protein levels. Our findings suggest that MMP9 may play significant role in aggressive behavior of breast cancer associated with local and brain metastases.

329 A Large-Scale Tissue Microarray Study Confirms the Prognostic Value of P-Cadherin Expression in Breast Cancer and Association with Her2+ and Basal Subtypes

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Background: P-cadherin (P-cad) is a calcium-dependent cell-cell adhesion glycoprotein in the adherens-type junctions, mainly promoting homotypic interactions in epithelium. P-cad expression is restricted to the myoepithelial cells in normal breast tissue, and aberrant staining has also been described in invasive breast tumors. Several small studies have reported that P-cad is a marker of poor outcome in breast cancer patients.
Design: A tissue microarray was constructed from 4,444 cases of invasive breast carcinoma linked to treatment and outcome information, and P-cad expression was evaluated using immunohistochemistry (IHC). Median follow-up was 12.5 years. The IHC-based definition of cancer subtypes was as follows: luminal (ER+ or PR+, HER2-), luminal/HER2+ (ER+ or PR+, HER2+), HER2+ (ER-, PR-, HER2+), and basal (ER-, PR-, HER2-, CK5/6+ or EGFR+). Clinical covariate and biomarker associations were assessed using contingency tables and significance of associations determined using Pearson's Chi-square or Fisher's exact test. Survival and relapse associations were visually assessed using Kaplan-Meier plots, with significance assessed using Logrank and Breslow tests and Cox proportional hazards regression analysis.
Results: P-cad was expressed in 50% (1875/3745) of cases, and P-cad+ patients showed significantly poorer short term (0-10 years) overall survival. P-cad staining was strongly associated with HER2+ and basal carcinoma subtypes (p<0.0005). The patients with P-cad+ tumors showed poorer disease-specific survival, distant relapse-free survival, loco-regional relapse-free survival and any relapse-free survival in univariable models (p<0.05). In a multivariable Cox model containing standard clinical covariates and tumor subtypes, P-cad did not show independent prognostic value. P-cad expression was positively associated with histologic grade, chemotherapy treatment, Ki67, epidermal growth factor receptor (EGFR), cytokeratin 5/6 (CK5/6), p53, YB-1 and HER2 expression (p<0.002), and negatively associated with age at diagnosis, ER, PR and Bcl-2 expression (p<0.0005).
Conclusions: This study shows the value of P-cad expression as a marker of poor prognosis in a large breast cancer series. P-cad positivity is associated with high-grade tumor subtypes and well-established markers of poor prognosis, and may represent a promising therapeutic target.

330 Biological Intrinsic Classification of Breast Cancer by Immunohistochemistry and qPCR Using Blocks from the NCIC MA.12 Trial

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Background: Gene expression profiling classifies breast cancer into biological intrinsic subtypes luminal A, luminal B, HER2-enriched (HER2-E), basal-like and normal-like. qPCR is the gold standard for gene expression profiling from tissue blocks but requires additional sample handling and complex data analyses. Immunohistochemistry (IHC) is a readily available and cost effective morphologic alternative, although lacks accuracy for some subtypes. We used tissue materials from clinical trial NCIC MA.12 with primary breast cancer and subtyped them using IHC and qPCR.
Design: Tissue microarrays were constructed using formalin-fixed paraffin-embedded cores from 472 patients in the the MA.12 trial. IHC for ER, PR, HER2, CK5/6, EGFR and Ki67 was used to define intrinsic subytpes using established criteria. Total RNA from 399 patients was sufficient for gene profiling with a 50 gene predictor (PAM50) by qPCR. In 354 cases, intrinsic subtyping into luminal A, luminal B, HER2-E and basal-like was obtained using both methods.
Results: Of the 472 cases in the tissue microarray, using IHC, 39% (n=190) were luminal A, 30% (n=143) luminal B, 7% (n=33) HER2-E, 17% (n=80) basal-like and 6% (n=26) unclassifiable. Of 399 cases with PAM50 qPCR expression profiles, 35% (n=141) were luminal A, 21% (n=85) luminal B, 18% (n=72) HER2-E, 21% (n=85) basal-like and 4% (n=16) normal like.

Sensitivity and specificity of immunohistochemical surrogate panels against expression profile as gold standard.

biologic intrinsic subtype by qPCR	definition by IHC	sensitivity	specificity
luminal A	(ER or PR)+, HER2-, Ki67 low	79%	85%
luminal B	(ER or PR)+, HER2+/-, Ki67 high	67%	76%
HER2-E	ER-, PR-, HER2+	35%	99%
basal-like	ER-, PR-, HER2-, (CK5/6 or EGFR)+	93%	99%

Conclusions: The immunohistochemical surrogate panel of ER, PR, HER2, CK5/6, EGFR and Ki67 can be used to classify breast cancer into groups that correlate with the molecular subtypes of luminal A, luminal B, HER2-E and basal-like. The established criteria identfies the basal-like group with high accuracy, while the identification of the HER2-enriched group was highly specific but not sensitive.

331 Mammaglobin Distinguishes Cutaneous Metastasis of Breast Cancer from Primary Cutaneous Adnexal Tumors

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Background: Distinction between cutaneous metastases of breast carcinoma and primary cutaneous adnexal tumors can be challenging. Immunohistochemistry (IHC) for estrogen and progesterone receptors (ER, PR) or for gross cystic disease fluid protein (GCDFP) offer little value due to overlapping immunophenotypes. Furthermore, a subset of breast cancers with aggressive behavior are negative for ER, PR and HER2 (so-called triple negative cancers). Mammaglobin is also a marker of breast cancer but its specificity in differential diagnosis with primary cutaneous adnexal tumors has not been formally established. It could be of particular value in the setting of triple-negative breast cancers.

Design: Mammaglobin (DAKO, 1:2) IHC was performed on whole sections of 16 cutaneous metastases of breast cancer, on tissue microarray sections of 190 primary breast cancers, including 118 triple negative cancers, and on whole sections of 64 primary cutaneous adnexal tumors (43 female, 21 male) with growth patterns simulating metastatic adenocarcinoma (14 sebaceous carcinoma, 7 microcystic adnexal carcinoma, 18 poroma, 12 syringoma, 5 desmoplastic trichoepithelioma, 3 adnexal adenocarcinomas NOS, 2 adenoid cystic carcinoma, and 3 adenocarcinoma of uncertain origin.) IHC was interpreted without knowledge of the morphologic diagnosis. Membranous/cytoplasmic expression was considered positive. Staining patterns were classified as strong/diffuse or weak/patchy.

Results: Mammaglobin was expressed in 10/16 (62.5%) cutaneous metastases of breast cancer, 23% of triple negative primary breast cancers, and 58% of ER positive primary breast cancers. Strong/diffuse expression was seen in 6/10 cutaneous metastases and weak/patchy in 4/10. Normal eccrine ducts were present in 41 skin specimens and 37 (78%) exhibited luminal/membranous mammaglobin expression. However, only 2/64 primary adnexal tumors were mammaglobin positive (both were male patients): one was an adenoid cystic carcinoma with strong/diffuse expression and one was a glandular neoplasm with poroid features showing weak/patchy expression. Overall, specificity of mammaglobin for breast cancer in this differential diagnosis was 96.9%; sensitivity was 62.5%.

Conclusions: Mammaglobin exhibited high specificity as a marker of cutaneous metastases of breast cancers and positive staining in a cutaneous glandular neoplasm should strongly raise consideration of breast origin even in the absence of ER,PR,HER2 expression. A negative stain result, however, is not conclusive since the sensitivity is moderate at best.

332 High Diagnostic Accuracy of FNAC Performed in a One Stop Clinic for the Diagnostic for Breast Lesions: A 3-Years Experience with 1822 Specimens

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Background: Fine-needle aspiration cytology (FNAC) is used extensively in the breast lesions diagnosis, but false-negative rates are a matter of concern. Immediate onsite evaluation of breast lesions, combining FNAC results with clinical and radiological data improves its diagnostic accuracy. The objective of this study was to evaluate the diagnostic accuracy of a large series of FNAC of breast lesions during the first 3-year period of the dedicated one-stop clinic, by comparing the FNAC results with the corresponding definitive histological examination outcome or the results of the radiological follow-up at 18 months.

Design: Data of consecutive patients (pts) whose lesions were prospectively characterized by ultrasonography (US) and diagnosed by FNAC in the one stop clinic of the Institute Gustave Roussy between May 2004 and March 2007. Histological verification by core-needle biopsy or surgery was systematically performed for lesions classified malignant or suspicious by cytopathology, and for benign lesions, when no perfect concordance between clinico-radiologic features and FNAC results (such as benign FNAC but BI-RAD 5) was found. For non-operated patients, follow-up consisted on US and/or mammography. For each cytopathological category, likelihood ratio (LR) was calculated as the ratio of the proportion of breast lesions with cancer and classified in a given cytopathological category to the proportion of breast lesions without cancer and classified in the same cytopathological category. Pts characteristics, radiological findings, cytopathological and histological results were extracted from the hospital computerized prospectively registered medical records.

Results: 1822 breast lesions (mean size 20 mm, BI-RAD ACR 1/2/3/4/5/ unknown: 10/96/471/459/777/9) in 1739 pts (mean age 56 years) were studied. FNAC was US-guided in 1115 lesions. Lesions were classified by FNAC as malignant in 842 (46%), benign in 771 (42%), suspicious in 154 (9%), and unsatisfactory in 55 (3%) cases. LR for malignancy were respectively estimated at 129 and 2,4 for lesions classified as malignant and suspicious in cytopathology, and reached respectively 0.4 and 0.036 in lesions unsatisfactory and benign in cytopathology.

Conclusions: Breast FNAC performed in a dedicated one-stop clinic with immediate on-site diagnosis represented a very efficient tool for triaging patients candidates for histological evaluation or for follow-up by imaging.

333 Epidermal Growth Factor Receptors and Topoisomerase II Alpha in Primary Adenoid Cystic Carcinomas of the Breast

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Background: Adenoid cystic carcinoma (ACC) is a rare, special subtype of breast carcinoma constituting approximately 0.1% of all breast carcinomas. Its expression profile (ER-, PR-) closely mimics that of the basal-like type of the breast carcinoma. Since there are no specific targeted therapies suitable for such tumors, research on the new potential drug targets is especially important.

Design: Twenty primary breast ACCs (19 females and 1 male) were studied for expression of epidermal growth factor receptor-1 (Her-1/EGFR), human epidermal growth factor receptor-2 (Her-2/neu), and topoisomerase II alpha (*TOP2A*) using immunohistochemical (IHC) and fluorescent in situ hybridization (FISH) methods.

Results: All tumors were uniformly negative for Her-2/neu protein, whereas EGFR protein was detected in the majority of cases (14/20, 70%). Topoisomerase II alpha was detected in 7/20 cases (35%) but exhibited a weak nuclear positivity in 5% or less of all tumor cells. *TOP2A* and *EGFR* gene alterations were rarely observed; only two cases exhibited gene deletions (TOP2A/CEP17 ratio 0.71, and EGFR/CEP7 ratio 0.70, respectively). No gene amplification was observed in any of the studied cases.

Conclusions: ACC of the breast is a typical triple negative, basal-like breast carcinoma with a common EGFR protein overexpression. Due to its *TOP2A* negativity patients with ACC of the breast are unlikely to respond to the antracycline based chemotherapy. Near uniform expression of EGFR in ACC should be further explored for targeted therapy.

334 Sentinel Node Biopsy in Early Breast Cancer: Does Tumor Burden in Sentinel Node Predict the Number of Positive Axillary Nodes?

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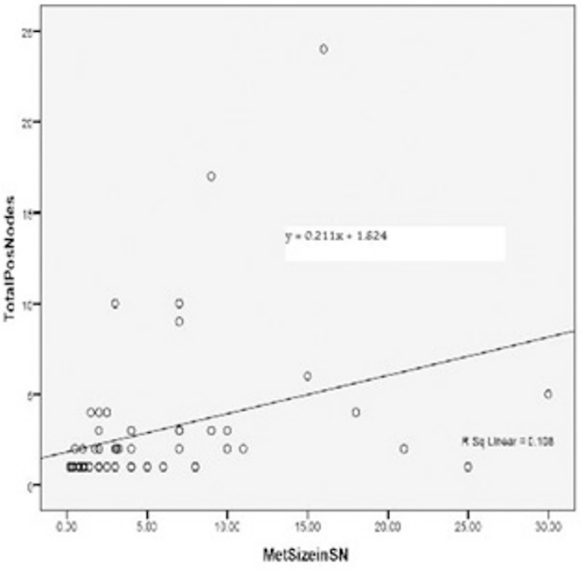
Background: The sentinel node (SN) in breast carcinoma is the first lymph node to which cancer is likely to spread from the primary tumor. Current practice dictates that most patients with a positive SN (micro and macro-metastasis) will undergo a complete axillary dissection. The SN biopsy alone is highly sensitive in predicting axillary lymph node status. Our study illustrates the correlation between tumor burden in the SN and the number of additional positive nodes in the axilla.

Design: 56 cases of pT1 breast cancers were studied to determine the size of metastatic tumor in the SN. The SN tumor burden was determined by measuring in millimeters the greatest dimension of the metastasis. The number of positive nodes in the corresponding axillary dissection was then recorded. A simple linear regression analysis was performed on the tumor size in the SN (independent) and the total number of positive lymph nodes in the axilla (dependent).

Results: There is a statistically significant correlation between the size of metastatic tumor in SN and number of positive axillary nodes in pT1 invasive breast cancers ($p<0.015$). Figure 1 shows the metastatic tumor size in SN on the X axis (MetsizeinSN) with total positive axillary nodes on the Y axis (TotalPosNodes).



Conclusions: In pT1 invasive breast cancer, the size of metastatic tumor in SN positively correlates with the number of positive axillary nodes. Therefore, in this model we can predict the number of total positive lymph nodes in a pT1 breast cancer using the size of the tumor burden in SN. This model has the capacity to further be adjusted for other factors including but not limited to age, race, and type of breast cancer. Based on our

evaluation, additional studies are indeed warranted which may require pooling of data from other pT1 data sets so that we may be able to accurately predict the outcome on a case by case basis for pT1 breast cancer.

335 Variations in Stromal Signatures in Breast Cancer Metastases

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Background: The tumor microenvironment (TME) plays an important role in tumor survival and growth but little is known about the degree of preservation between different stromal response patterns found in primary tumors and their metastases. We have previously identified gene signatures for two distinct stromal response patterns in breast carcinoma of fibroblast (aka DTF) and macrophage response (aka CSF1) and found them to be correlated with clinicopathologic features including outcome.

Design: To determine whether this signature is conserved between primaries and metastases, we examined the DTF fibroblast and CSF1 macrophage response signatures between matched breast cancer primary and metastases on 49 cases and matched colon cancer primary and metastases on 15 cases, all represented on a tissue microarray. Four previously established markers (FCGR3a, FCGR2a, CTSL1, and CD163) were used for the CSF1 macrophage response and 5 five markers (SPARC, VCAN, CDH11, SDC1 and MMP11), derived from the core gene set of the DTF fibroblast stromal signature, were used for the DTF fibroblast stromal signature. To validate our immunohistochemistry findings, we examined the stromal signatures in a gene expression profiling data set using published gene expression microarray data from a study comparing 15 primary breast tumors and their matched lymph node metastases.

Results: In both breast and colorectal cancer, there was a significant, positive correlation between the CSF1 macrophage signature in the primary tumors and the matched lymph node metastases as assessed by immunohistochemical markers. No such correlation was observed for the DTF fibroblast signature. The CSF1 macrophage positive correlation was confirmed by analysis of the gene expression microarray dataset of 15 primary and matched metastatic breast cancers.

Conclusions: The variations of these stromal reaction patterns from the primary to the metastasis shed lights on the relationship between the neoplastic cells and the non-neoplasic cells in the TME. The preservation of the CSF1 macrophage response pattern in metastases suggests a possible therapeutic target in these instances.

336 Prognostic Factors of Primary Invasive Mammary Neuroendocrine Carcinoma: *Clinicopathologic Analysis of 74 Cases*

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Background: Primary Neuroendocrine carcinoma (NEC) of the breast is an uncommon neoplasm. It was identified more than four decades ago, but its diagnostic criteria was not well defined till 2003, when WHO histologic classification of the breast tumors define this tumor having >50% of the tumor cells expressing neuroendocrine markers. The biological behaviour and prognostic factors of this type of tumor is not fully studied.

Design: Seventy-four mammary NEC cases from the Surgical Pathology file at The University of Texas M.D. Anderson Cancer Center were retrieved and reviewed, and the diagnoses were confirmed based on the positive immunohistochemical reaction to neuroendocrine marker synaptophysin and/or chromogranin in >50% of the invasive tumor cells. Clinicopathologic features and outcome data of these cases were obtained.

Results: Univariate analyses showed that large tumor size, high nuclear grade, presence of lymphovascular invasion in the primary tumor, and regional lymph node metastasis were adverse prognostic factors for distant recurrence-free survival; and that large tumor size, high nuclear grade, high Ki-67 proliferation index and regional lymph node metastasis were adverse prognostic factors for overall survival. Multivariate analysis revealed that presence of lymphovascular invasion and regional lymph node metastasis were independent adverse prognostic factors for distant recurrence-free survival; and that Ki-67 proliferation index was an independent prognostic factor for overall survival. Interestingly, this study also found that histologic grading system based on the assessment of tubule/gland formation, nuclear pleomorphism and mitotic counts, is not prognostically significant in predicting distant recurrence-free survival and overall survival by both univariate and multivariate analyses.

Conclusions: This study suggest that NEC is a distinct clinicopathologic entity. Ki-67 proliferation index is a valuable prognostic marker in this type of tumor and should be routinely evaluated.

337 The Molecular Underpinning of Lobular Histological Growth Pattern: A Genome-Wide Transcriptomic Analysis of Invasive Lobular Carcinomas and Grade- and Molecular Subtype-Matched Invasive Ductal Carcinomas of No Special Type

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Background: The aims of this study were to determine the transcriptomic characteristics of lobular carcinomas (ILCs) and to define the genome-wide transcriptomic differences between classic ILCs and pleomorphic lobular carcinomas (PLCs).

Design: To define the transcriptomic characteristics of ILCs, minimising the impact of histological grade and molecular subtype on the analysis, we subjected a series of ILCs, including classic and pleomorphic variants, and grade- and molecular subtype-matched invasive ductal carcinomas (IDCs) to genome-wide gene expression profiling using oligonucleotide microarrays. Validation of selected genes identified as differentially expressed between ILCs and IDCs and between ILCs and PLCs was performed in an independent cohort of grade- and molecular subtype-matched ILCs and IDCs using real time RT-PCR.

Results: Hierarchical clustering analysis demonstrated that ILCs formed a separate cluster and a supervised analysis revealed that 5.8% of the transcriptionally regulated genes were significantly differentially expressed in ILCs compared to grade- and molecular subtype-matched IDCs. ILCs displayed down-regulation of E-cadherin and of genes related to actin cytoskeleton remodelling, protein ubiquitin, DNA repair, cell adhesion, TGF-beta signalling, and upregulation of transcription factors/ immediate early genes, lipid/ prostaglandin biosynthesis genes, and cell migration-associated genes. Supervised analysis of classic ILCs and PLCs demonstrated that <0.1% of genes were significantly differentially expressed between these tumour subtypes. Quantitative RT-PCR analysis of the independent validation cohort confirmed that ANKRD28 and AFF1 were significantly expressed at higher levels in ILCs than in grade- and molecular subtype-matched IDCs and that HIF1A was expressed at higher levels in PLCs than in ILCs.

Conclusions: Our results demonstrate that ILCs differ from grade- and molecular subtype-matched IDCs in the expression of genes related to cell adhesion, cell-to-cell signalling and actin cytoskeleton signalling. Classic ILCs and PLCs are remarkably similar at the molecular level and should be considered as part of a spectrum of lesions.

338 Breast Cancer Molecular Subtype Classification: A Comparison of Three Microarray-Based Single Sample Predictors

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Background: Microarray gene expression profiling has led to the identification of five molecular subtypes of breast cancer: luminal A, luminal B, HER2, normal breast-like and basal-like. Three single sample predictors (SSPs) have been described by the proponents of this taxonomy for the identification of molecular subtypes. The aim of this study was to determine the agreement between these SSPs in the identification of breast cancer molecular classes.

Design: Microarray-based SSPs were applied to one in-house and three publicly available breast cancer microarray datasets based on the methods described by the proponents of the molecular taxonomy. Analysis of agreement between each pair of SSPs was performed for the whole classification system and for each molecular subtype individually in each cohort. Hazard ratios for outcome for each molecular subtype according to each SSP in each cohort were calculated. For the in-house dataset, HER2 status was defined by immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH).

Results: A fair-to-substantial agreement between each pair of SSPs in each cohort was observed (Kappa scores ranging from 0.238 to 0.740). The proportion of cases classified as basal-like subtype in each cohort was consistent regardless of the SSP employed, however the percentages of the remaining molecular subtypes varied significantly. Of the five molecular subtypes, only basal-like cancers consistently showed an almost perfect agreement (Kappa scores >0.810). Most importantly, the significance of the associations with outcome of each molecular subtype other than basal-like and luminal A varied significantly depending on the SSP employed. The identification of HER2-positive tumours by SSPs was unreliable, given that one SSP assigned only 54% of the IHC/FISH HER2-positive cases to the HER2 molecular subtype, whereas the other two SSPs assigned all IHC/FISH HER2-positive cases to the luminal B subtype.

Conclusions: Basal-like cancers can be reproducibly identified by microarray-based gene expression profiling. The classification of breast cancers into molecular subtypes shows considerable variation depending on the SSP used. Before molecular classification by microarray profiling can be incorporated in routine clinical practice and treatment decision-making, accurate definitions of the methods used to identify the molecular subtypes of breast cancer should be standardised and rigorously validated.

339 Expression of Id4, a BRCA1 Downregulator, in Triple Negative Breast Cancer

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Background: Somatic *BRCA1* mutations are rare in sporadic breast cancer (BC). Id4, a transcription factor downregulating *BRCA1 in vitro*, could contribute to epigenetic *BRCA1* inactivation in sporadic BC. To test this hypothesis, we compared the expression of Id4 in a series of triple negative breast carcinoma (TN) versus control (C) BC, and correlated the results with basal markers and clinical follow-up (F/U).

Design: We assessed the morphology of 54 TNBC and 105 CBC treated at our center (2002-4). Immunostains for Id4, CK14, calponin and p63 were performed on a representative section of TNBC and on tissue microarrays of CBC. Fisher's exact test and t-test were used for statistical analysis.

Results: Table 1 summarizes the clinicopathologic characteristics of our cases. All TNBC were invasive ductal (12 with a large central acellular zone (LCAZ), 5 apocrine, 1 with medullary features,1 matrix producing). CK14 was positive in 24 (44%) TNBC. Id4 occurred in 37 TNBC (69%) and involved >50% of tumor cells in 14 (26%), 5-50% in 14 (26%) and <5% in 9 (17%). Id4 expression in >5% of tumor cells correlated with LCAZ (36% vs 8%, p=0.021) and CK14 positivity (71% vs 15%, p=0.0001). Ten of the 13 TNBC with distant metastases were Id4+ (p=0.0001). In contrast, only 4 (4%) CBC had rare Id4+ cells (p=0.0001) and all 4 CBC with distant metastases were Id4-.

		TN (n=54)	Control (n=105)	p
Median age		55	51	
Size (cm)		2.7 (0.3-28)	2.6 (1.1-18.5)	
Grade	1	1 (2%)	16 (15%)	0.006
	2	12 (22%)	39 (37%)	0.033
	3	41 (76%)	44 (42%)	0.0002
	N/A	0	6 (6%)	
LCAZ		12	0	0.0001
LN met		25 (46%)	68 (65%)	0.028
Treatment	Chemotherapy	50 (93%)	86 (82%)	
	Hormone therapy alone	0	14 (13%)	
	Surgery alone	4 (7%)	5 (5%)	
F/U		n=51	n=103	
Median F/U (y)		4	5	
Mets		13 (25%)	6 (6%)	0.001
	Brain	4 (8%)	2 (2%)	
	Lung	5 (10%)	3 (3%)	
	Bone	4 (8%)	4 (4%)	
	Liver	7 (14%)	0	
Id4		37 (69%)	4 (4%)	0.0001
CK14		24 (44%)	4 (4%)	0.0001

Conclusions: Id4 expression is frequent in TNBC and correlates with basal phenotype and distant metastases. Evaluation of Id4 in additional cases and correlation with BRCA1 status is in progress. The results of our study could help to identify TNBC with sporadic BRCA1 inactivation, suitable for treatment with PARP inhibitors.

340 Morphology and Immunophenotype of Ductal Carcinoma In Situ in Triple Negative Breast Cancer

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Background: Information on precursor lesions associated with triple negative breast carcinoma (TN) is limited. We evaluated the morphology of DCIS adjacent to TN and compared its immunoprofile with that of the coexisting invasive component.

Design: We reviewed cases of TN and identified 54 in which DCIS appeared to be present based on morphology. For each tumor, we selected a representative slide/block with DCIS and performed immunoperoxidase stains for calponin, p63, CK5/6, CK14 and Id4.

Results: All patients were women, with median age 55 y (range 29-84). All TN were invasive ductal carcinoma, including 47 NOS and 5 apocrine; one had medullary features, one was matrix producing. The average tumor size was 2.7 cm (range 0.3-28.0). Forty-one TN (76%) had modified Bloom-Richardson grade 3, 12 (22%) grade 2, and 1 (2%) grade 1. Myoepithelial stains verified DCIS in 41 (76%) cases. Solid nests of invasive carcinoma (10) or lymphovascular invasion (2) mimicked DCIS in 12 cases, a minute focus of DCIS was lost in deeper sections in one case. DCIS was of solid type in 26 (63%) cases, cribriform in 4 (10%) and mixed (solid, cribriform, micropapillary or papillary) in 11 (27%). Nuclear grade was high in 33 (80%) cases, intermediate in 8 (20%). Necrosis was present in 33 DCIS (80%), and was extensive in 11 (27%), moderate in 15 (37%), minimal in 7 (17%). Calcifications in DCIS occurred in 14 (34%) cases. DCIS was positive for basal cytokeratins in 14 (34%) cases: 8 (20%) were CK14+/CK5/6+; 3 (7%) CK14+/CK5/6-; 3 (7%) CK14-/CK5/6+. P63+ cells were seen in 2 DCIS, but only one showed focal positivity for CK14 and CK5/6. Id4, a downregulator of BRCA1 inversely correlated with ER, was detected in 24 of 38 (63%) evaluable DCIS and in all of the adjacent TN. Concordant positive or negative expression in DCIS and adjacent TN was observed for CK14 in 33 (80%) cases, for CK5/6 in 30 (73%) and for Id4 in 36 (95%).

Antigen	DCIS	Invasive
CK14 and/or CK5/6	14 (34%)	19 (46%)
CK14-/CK5/6-	27 (66%)	22 (54%)
p63	3 (7%)	3 (7%)
Id4	24 (63%)*	28 (68%)

*only 38 DCIS were evaluable

Conclusions: DCIS associated with TN typically has high nuclear grade and necrosis, but carries calcifications in only about a third of cases. When present, DCIS shows basal immunophenotype, similar to that of the adjacent TN, supporting a role as a precursor lesion. Id4 expression in DCIS associated with TN suggests that sporadic BRCA1 inactivation may occur in this type of tumors.

341 Distinction of Chemotherapy-Induced Epithelial Atypia from Minimal DCIS with Chemotherapy Effect in HER2 Positive Patients Using HER2 Immunostaining

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Background: Chemotherapy can induce nuclear and cytoplasmic alterations in both neoplastic and benign breast epithelium. This can create diagnostic challenges in distinguishing minimal volume DCIS (defined as minimal duct/lobular expansion and minimal intraductal proliferation) from non-neoplastic epithelium. Two problematic settings are 1.) evaluating margins in neoadjuvant treated specimens and 2.) evaluating core biopsies of new lesions in patients with prior adjuvant treatment. In patients with untreated HER2 positive cancer, the invasive and in situ components are typically concordant by immunoexpression (IHC) while benign epithelium is negative. Thus, HER2 IHC could potentially be used to detect and distinguish minimal residual DCIS following chemotherapy from benign treatment atypia.

Design: We evaluated 203 patients with neoadjuvant chemotherapy-treated breast cancer to identify those with HER2 positive cancers on pre-treatment biopsy. Of this subset, 26 had residual pure DCIS and HER2 IHC (CB11, Novocastra, 1:200) was performed on the post-treatment specimens. Residual DCIS was classified as minimal DCIS if the involved ducts/lobules were not expanded when viewed at low magnification. DCIS was further classified by degree of nuclear atypia, by degree of atypical apocrine change, and by degree of obscuring healing changes (atypical ductal epithelium with obscuring chronic inflammation, histiocytes and ductal fibrosis and/or dilation).

Results: HER2 was strongly expressed in 22/26 residual DCIS cases, including 11/12 with minimal DCIS, most of which consisted only of single atypical cells with nucleocytomegaly interspersed between normal ductal epithelium. Of the HER2 negative residual DCIS cases, 3/4 showed classic low grade DCIS without treatment effects and 1/4 was minimal DCIS. All 8 cases with atypical apocrine change were HER2 positive. All 8 cases with features suspicious for healing DCIS contained HER2 positive cells embedded in the intraductal inflammatory reaction. The extent of minimal DCIS was underestimated on H&E stain compared to HER2 IHC in 5/26 cases. Among 10 lumpectomies with minimal residual DCIS requiring re-excision, 6/10 contained DCIS in the re-excision.

Conclusions: Positive HER2 IHC distinguishes minimal DCIS from benign atypia in chemotherapy treated HER2+ patients. H&E slides may underestimate extent of DCIS in this setting and attention should be aimed at atypical apocrine changes and healing ducts when evaluating neoadjuvant margins or core biopsies of new post-treatment lesions.

342 Ki-67 Image Cytometric Quantitation and Oncotype Dx Recurrence Score in Estrogen Receptor-Positive Breast Cancer

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Background: The prognosis of patients with breast cancer has traditionally been determined by assessing clinicopathologic features such as lymph node metastasis, tumor size, and histologic grade. Recently, the 21-gene assay Oncotype Dx has been used in lymph node-negative, estrogen receptor (ER)-positive breast cancer to refine prognosis and direct therapy. Its utility, however, is limited by its cost, proprietary nature, and lengthy turnaround time. Ki-67 is a proliferation marker whose expression can rapidly and inexpensively be characterized by immunohistochemistry. Our aim is to correlate the expression of Ki-67 with the Oncotype Dx Recurrence Score (RS) and other prognostic indicators.

Design: ER-positive invasive breast carcinomas from 130 patients with Oncotype DX RS testing over a 2 year period were selected. Representative 5 micron tumor sections were stained with MIB1 (Dako, Carpinteria, CA), a monoclonal antibody that reacts against Ki-67. MIB1 nuclear staining was quantitated using the Automated Cellular Imaging System III (Dako). The percentage of MIB1-positive cells was assessed in ten random 40x fields, and the average was used to rate MIB1 staining as low (<10%), intermediate (10-20%), or high (>20%). MIB1 staining was compared with the Oncotype Dx RS (low [<18], intermediate [18-30], or high [>30]) and clinicopathologic parameters including grade, angiolymphatic invasion, lymph node metastasis, and tumor size.

Results: MIB1 staining was low in 36.9% (48/130) of cases, intermediate in 36.9% (48/130), and high in 26.1% (34/130). There were significant associations between MIB1 staining and Oncotype Dx RS (P=0.02; Table 1), grade (P<0.001), and angiolymphatic invasion (P=0.01). Lymph node metastasis and tumor size were not associated with MIB1.

Table 1. Correlation between MIB1 and Recurrence Score

	Low RS	Intermediate RS	High RS	P-value
Low MIB1	64.6% (31/48)	33.3% (16/48)	2.1% (1/48)	
Intermediate MIB1	47.9% (23/48)	39.6% (19/48)	12.5% (6/48)	
High MIB1	35.3% (12/34)	35.3% (12/34)	29.4% (10/34)	P=0.02

Conclusions: MIB1 staining detects expression of the proliferation marker Ki-67. Our study demonstrated increased Ki-67 expression in high grade cancers and in tumors with angiolymphatic invasion. Additionally, we identified a significant association between Ki-67 expression and Oncotype Dx RS. This observation suggests that Ki-67, in conjunction with other readily available prognostic markers, may provide useful prognostic information when the Oncotype Dx RS is unavailable.

343 Development of a Population Based Model for Progression of DCIS to Invasion

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Background: Understanding the progression of ductal carcinoma in situ (DCIS) to invasive breast cancer (IBC) is critical to effective treatment and early prevention of subsequent invasion. Clinical observations report that nearly 67% of DCIS cases have already progressed to IBC at time of diagnosis. Our goal is to develop a mathematical model that predicts this observation.

Design: We developed a linear compartmental model with transition rates for normal mammary epithelium to DCIS of any grade and then from high grade (HG), intermediate grade (IG) and low grade (LG) DCIS to IBC. The model consists of coupled ordinary differential equations.

Results: We assume time interval between initiation of DCIS and diagnosis is the average time between mammograms based on current screening recommendations, 1.3 years. We fit rate constants for progression to IBC from published data (literature and SEER statistics) and apply it to an assumed distribution of DCIS by grade (40% HG, 40% IG and 20% LG). We predict that 7.1% of DCIS will have co-existing IBC within 1.3 years. We also fit a rate of progression for LG DCIS and then scaled it for IG and HG as a function of the relative proliferation index for the different grades as measured in our laboratory. This results in a prediction of 43% of cases of DCIS having co-existing IBC. If we consider DCIS to be 100% HG, then we predict 65% has invaded after 1.3 years.

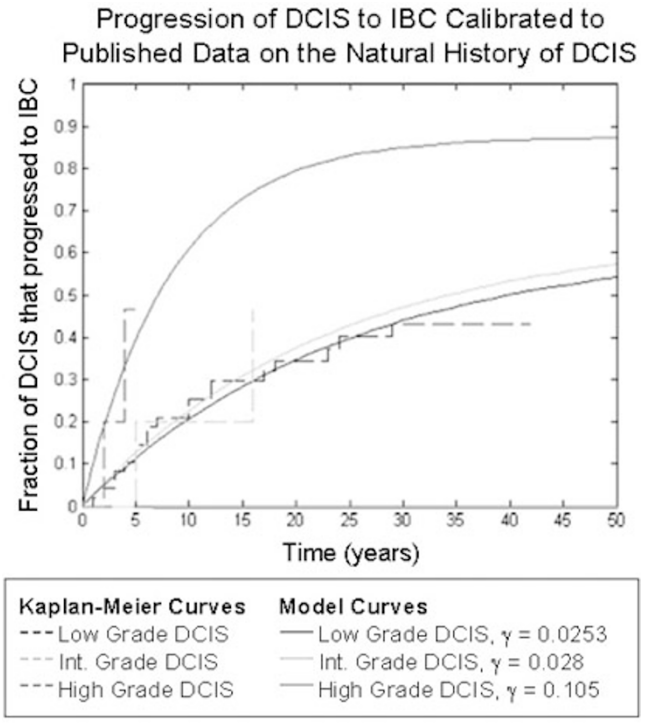

Figure 1. Kaplan-Meier curves and model curves, which are obtained from data on the natural history of DCIS

Conclusions: We conclude that the progression of DCIS to IBC cannot be fully explained with a simple transition model. Our findings suggest that there are two rates for transition from DCIS to IBC, a fast one that nearly reaches 90% of its steady state value within 1 year and is independent of grades, and a rate constant that is dependent upon grade with LG and IG DCIS having the slowest rates of transition.

344 The Relationship between Recurrence Score of 21-Gene Assay and Clinicopathological Factors of Breast Cancer

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Background: Oncotype DX has been increasingly used to aid adjuvant treatment decisions in breast cancer management. In the current study, we sought to investigate the relationship between Oncotype DX recurrence scores (RS) and clinicopathological features in invasive breast carcinomas.

Design: We identified 100 infiltrating carcinomas (91 IDC and 9 ILC) that had Oncotype DX performed from our departmental file and analyzed the relationship between RS and clinicopathological factors. Histologic grades were defined by a consensus results from our breast pathologist group, ER and PR were recorded as Allred scores. HER2 was scored as positive if >30% of tumor cells showed 3+ membrane staining. EGFR was designated as positive if any tumor cells showed 1+ positive stain. Any strong cytoplasmic stain was considered as positive for CK5/6. Ki-67 was recorded as % of nuclear stain. The definitions for each molecular subtype were based on the expression of ER, HER2, EGFR and CK5/6.

Results: Among the 100 cases, 60, 34 and 6 cases belonged to low, intermediate, high risk group, respectively. All tumors in low RS group fell into grades 1-2 tumor; while grade 3 tumors with a RS ranging between 20-54. The p-value for tubule formation, nuclear pleomorphism, and mitosis were 0.1412, 0.1209, and 0.1178, respectively. The mean Allred scores for PR were significantly different (p=0.0003) among low, intermediate, and high RS groups, 7.58, 6.65 and 2.22, respectively. Tumors with a PR Allred score 4 or less had RS approaching or in intermediate or high risk groups (between 16 and 54), Tumors with a 2+(confirmed by FISH) or 3+ of HER2 level had RS between 23 and 54, and with a p-value of 0.0320. 100%, 91% and 50% of low, intermediate, and high RS groups belonged to Luminal A subtype (ER+, HER2-). Ki-67 was not significantly different among these 3 groups (10.83%, 15.03% and 7.33%, respectively), so did patient age (56.27, 53.29 and 60.33, respectively) and tumor size (1.84cm, 2.01cm and 1.42cm, respectively).

Conclusions: Nottingham grade, expression of PR and HER2 should be taken into consideration when choose cases for 21-gene assay in ER positive tumors. Tumors that are grade 3, PR Allred score 4 or less, and HER2 2+ or 3+ are likely to have a higher RS, and may not be needed for 21-gene assay testing.

345 Histological Grading of Breast Carcinoma: Variability between Breast Subspecialty Pathologists and General Pathologists

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Background: The Nottingham grading system is the most commonly used grading scheme for breast carcinomas and is based on a combined score for tubule formation, nuclear cytology, and mitotic rate. While guidelines of the grading system attempt to define criteria for the score within each category, the assignment of these scores by pathologists remains subjective. With subspecialty practice has become more popular; we sought to investigate the agreement in histologic grading between general pathologists and breast pathologists.

Design: Ninety-seven breast carcinomas were pulled from the files at our departmental file and reviewed first independently by five pathologists in the breast subspecialty group and then re-examined for a consensus if there was a disagreement. The Nottingham score (3-9) and grade (1-3) were compared between the originally reported grade given by general pathologists and the consensus grade from the breast pathologists for each case.

Results: Among the 97 cases, the complete agreement was 71 (73.2%) for histologic grade, 76 (78.6%) for tubule formation, 55 (56.7%) for nuclear grade, and 69 (71.1%) for mitotic count. There was good agreement between the consensus grade and the originally reported grade (kappa 0.65), as well as the consensus total score and the originally reported score (kappa 0.62). Comparing the individual scores for tubules, nuclear cytology, and mitotic figures showed very good agreement for the tubule score (kappa 0.73), but less agreement for the nuclear score (kappa 0.39) and mitotic rate (0.35). The agreement for histologic grading among 5 breast pathologists were fairly good, with 49 cases with complete agreement, 18 cases with agreement on 4 pathologists, 29 cases with 3 pathologists. Only one case had discrepancy among all 5 pathologists (G1 by 2, G2 by 2, and G3 by 1), which might due to multiple slides with tumor from this case.

Conclusions: While it is reassuring that there is very good agreement between the final grade and the score that grade is based upon, there remains a lack of reproducibility especially in the nuclear and mitotic scores. Subspecialty practice may not have an effect on grading consistency.

346 Fibroblast-Like Stromal Response Is Host-Dependent While Macrophage-Associated Stromal Response Is Tumor-Dependent: A Study of Stromal Response in Paired Breast Carcinomas from Patients with Dual Primaries

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Background: Recent work has demonstrated that stromal response genes correlate with tumor grade and patient survival. In breast cancer, the desmoid tumor fibromatosis (DTF)-like fibroblastic response is associated with lower grade and increased survival, whereas CSF1 macrophage response is associated with higher grade and decreased survival. It is unclear whether stromal response in these instances arises from a host response to the tumor, or whether the cancer induces a specific set of stromal responses. We explored this question by comparing stromal response signatures in patients with dual breast primaries.

Design: Seventeen patients with dual primaries were included in this study, with a total of 34 carcinoma cases analyzed. Immunohistochemical markers included previously defined DTF fibroblast expression signature genes (cadherin 11, MMP 11, CSPG2, CD138, osteonectin) and CSF1 macrophage response signature genes (cathepsin L, CD163, CD16, CD32). Analysis using paired mutation and Wilcoxon-Mann-Whitney test was performed to determine the relationship of stromal response arising from the patients' dual primaries.

Results: Permutation analysis demonstrated a significant, positive correlation for expression of DTF markers between the paired primary breast carcinomas (p=0.00268), whereas expression of CSF1 markers was not correlated between the 2 primaries (p=0.514). Of the individual markers, MMP11 (p=0.0052), cadherin 11 (p=0.0421), cathepsin L (p=0.00315) exhibited significant shared expression between the patients' 2 primaries. There was no correlation between histologic type (ductal vs lobular) and expression of DTF and CSF markers (p=0.975 and p=0.454). Similarly, there was no correlation with tumor location (p=0.100).

Conclusions: In studying the relationship between tumor and host response, we identified two sets of stromal signatures with divergent associations: one set of markers appears to be consistently expressed in a given patient regardless of tumor type (DTF fibroblast expression signature), while a second set of markers appears to be dependent on the inciting tumor (CSF1 macrophage expression signature). As the DTF fibroblast response has been associated with improved survival, this finding suggests that a subset of patients may generate a host response to tumors that favors good outcome.

347 Tumor Size, Nuclear Grade and HER2 Positivity Predict Lymph Node Metastasis in Breast Carcinomas That Have Lymphovascular Invasion

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Background: Lymphovascular invasion in breast carcinomas has been shown to be a poor prognostic factor, at least partly due to its frequent association with lymph node metastasis. In current study, we sought to investigate predictive clinicopathologic factors for lymph node metastasis in breast carcinomas that have lymphovascular invasion.

Design: Two hundred ninety-one cases of breast carcinomas with lymphovascular invasion between 1997 and 2008 were identified from our departmental file. Clinicopathological information including age of patients, tumor type, tumor size, histologic grade, nuclear grade, lymph node status, and expression of ER, PR and HER2 were recorded. Statistical analyses were performed to compare lymph node positive and negative tumors.

Results: Among the 291 cases, 106 cases (36.4%) had negative lymph nodes and 185 (63.6%) cases had positive lymph nodes. In node negative tumors, 99% and 1% were ductal and lobular carcinoma, respectively. In node positive tumors, 95% and 5% were in ductal and lobular carcinomas. Although there was a trend of lobular carcinoma being more frequently associated with nodal metastasis, no significant difference was seen

between these two groups (p=0.2136). The mean ages were 57.43 for node negative tumors and 55.50 for node positive tumors (p=0.2181). There was a significant difference of tumor size between node negative (2.27cm) and node positive tumors (3.55cm), with a p-value less than 0.0001. While we did not observed a significant different for histologic grade between these two groups (p=0.4018); there was a significant difference for nuclear grade between these two groups (p=0.018). There was also difference in HER2 over-expression between these two groups (p=0.0595), but no difference was observed in the expression of ER (p=0.4379) and PR (p=0.6648) between these two groups of tumors.

Conclusions: Tumor size, nuclear grade, and HER2 positivity predict lymph node metastasis in breast carcinomas that have lymphovascular invasion. Tumor type, patient age, histologic grade, and expression of ER and PR are not predictive.

348 Fibroepithelial Lesion with Cellular Stroma: Topoisomerase 2 Is a Helpful Marker To Differentiate Fibroadenoma from Phyllodes Tumor on Needle Core Biopsy

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Background: The differential diagnosis of fibroepithelial lesions with cellular stroma (FELCS) in core needle biopsy (CNB) specimens ranges from fibroadenoma (FA) to phyllodes tumor (PT). The management of these two lesions is different. We intended to explore possible morphologic and immunohistochemical (IHC) parameters that may predict the final diagnosis on the excisional biopsy.

Design: A series of FELCS cases diagnosed on CNB with matching excisional biopsy were retrieved from our files between 2003 and 2009. The following histologic parameters were recorded: stromal cellularity (1, 2, or 3), stromal cell atypia (1, 2, or 3), stromal cell mitosis per ten high power fields, stroma overgrowth, infiltrative edge, stromal cellularity enhanced at epithelium and leaf-like pattern. Patients' age and tumor size were also recorded. The following IHC stains were performed on CNB: KI-67 (clone MIB-1), p53 (clone DO7) and Topoisomerase 2 (TOPO2) (clone EP1054Y). Percentage of positive cells was recorded. Fisher's exact test and Wilcoxon non-parametric test were used for statistical analyses.

Results: The table below illustrates all the findings.

Histologic, Clinical and IHC Finding

	FA (n=8)	PT (n=12)	p value
Age (y), median (range)	42 (35-48)	47 (30-82)	NS
Size (cm), median (range)	1.35 (0.5-2.5)	3.45 (0.5-15)	0.022
Stromal cellularity			
1 or 2	7 (87.5)*	8 (66.7)	NS
3	1 (12.5)	4 (33.3)	NS
Stromal cell atypia			
1	6 (75.0)	5 (41.7)	NS
2 or 3	2 (25.0)	7 (58.3)	NS
Stromal cell mitosis			
No	6 (75.0)	8 (66.7)	NS
Yes	2 (25.0)	4 (33.3)	NS
Stromal overgrowth			
No	5 (62.5)	6 (50.0)	NS
Yes	3 (37.5)	6 (50.0)	NS
Infiltrative edge**			
No	8 (100.0)	6 (66.7)	NS
Yes	0 (0.0)	3 (33.3)	NS
Stromal cellularity enhanced at epithelium			
No	8 (100.0)	11 (91.7)	NS
Yes	0 (0.0)	1 (8.3)	NS
Leaf-like pattern			
No	6 (75.0)	5 (41.7)	NS
Yes	2 (25.0)	7 (58.3)	NS
Median (range) Ki-67 (%)	5 (0-20)	10 (1-50)	NS
Median (range) p53 (%)	2.5 (0-90)	20 (0-80)	NS
Median (range) TOPO2 (%)	0 (0-2)	2 (0-10)	0.037

* No. (%), ** n=9 for PT

Conclusions: Larger clinically measured tumor size and presence of Topoisomerase 2 staining can predict PT on CNB.

349 Breast Hormonal Receptors Test Should Be Repeated on Excisional Biopsies after Negative Core Biopsy

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Background: Accurate estrogen receptor (ER) and progesterone receptor (PR) results are important for therapeutic decision making for patients with breast carcinoma. The purpose of this study was to assess the concordance of breast cancer immunohistochemical receptor assays on core biopsy and surgical specimens.

Design: We identified 176 patients whose core biopsy was performed either at Roswell Park Cancer Institute (RPCI) or at an outside facility between 2007 and 2009. Surgical specimens were processed in RPCI. ER and PR, for biopsies and excisions, were scored using Allred scoring system. While biopsies were processed in 12 different laboratories, stained in 5 different laboratories using 3 different vendors, the excisional biopsies were processed and stained in RPCI using one vendor (Dako). While the following antibodies were used for ER, 1D5, 6F11 and SP1, the following antibodies were used for PR, PgR636, 16 and 1E2 from Dako, Leica and Ventana respectively. Correlation of scores of biopsies with matching excision was analyzed using Spearman correlation coefficient test.

Results: Seventeen (9.7%) patients were biopsied in RPCI and 159 (90.3%) patients in an outside facility. While there were 141 (80.1%) cases positive for ER and 118 (67%) cases positive for PR for the core biopsy, there were 143 (81.3%) cases positive for ER and 130 (73.9%) cases positive for PR for the excision. Concordance for ER and PR was seen in 93% and 89.8% respectively. Table illustrates the concordance between biopsy and excision for both markers based on vendors. Spearman correlation coefficient between biopsy and excision was 0.75 for ER and 0.79 for PR (p<0.0001 each).

Table: Comparison of ER and PR between biopsy and excision based on Vendors.

	Dako (No. 23)	Leica (No. 124)	Ventana (No. 29)	P value
ER: BX+EX+	16 (69.6)*	100 (81.3)	20 (66.7)	0.11
ER: BX+EX-	0 (0)	3 (2.4)	2 (6.7)	
ER: BX-EX+	3 (13)	3 (2.4)	1 (3.3)	
ER: BX-EX+	4 (17.4)	17 (13.8)	7 (23.3)	
PR: BX+EX+	13 (56.5)	85 (69.1)	17 (56.7)	0.48
PR: BX+EX-	0 (0)	2 (1.6)	1 (3.3)	
PR: BX-EX+	2 (8.7)	11 (8.9)	2 (6.7)	
PR: BX-EX+	8 (34.8)	25 (20.3)	10 (33.3)	

* Number (percentage); BX, biopsy; EX, excision

Conclusions: Although there was no uniformity in biopsies processing or staining, practically speaking, ER and PR should be repeated on the excisional biopsies for patients whose core biopsies have negative hormonal receptor.

350 Tissue Factor Expression in Triple-Negative Breast Carcinomas

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Background: Tissue factor (TF) is expressed in a variety of tumor cells and has been linked to cellular signaling, angiogenesis, and tumor progression. However, its role in human cancer is not fully known. Recently, upregulation of TF has been linked to expression of epidermal growth factor receptor (EGFR), a prognostic factor of breast cancer. Triple-negative (ER, PR and HER-2) breast carcinomas (TNBC) belong to a subgroup of breast cancer with aggressive clinical behavior and poor prognosis.

Design: Forty-five cases of TNBC diagnosed from 2003 to 2008 were retrieved from the archive of the Department of Pathology of Temple University. Adequate tissue was available in 41 cases that formed the basis of this study. All patients were female with an age range from 32 to 81. Immunohistochemistry for CK5/6, EGFR and TF was performed, and results were scored as positive (tumor cells stained) or negative (no tumor cell stained). Basal-like carcinoma (BLC) was defined as a TNBC positive for CK5/6 and/or EGFR.

Results: 20 (49%) of patients presented with regional lymph node metastasis, 9 (22%) demonstrated distal metastasis and 24 (59%) had advanced clinical stage (III/IV). All cases were invasive ductal carcinoma (IDC), except for one adenoid cystic carcinoma. 36 of the 40 IDC were histologically high grade and the remaining 4 were intermediate grade. BLC was identified in 36 of the 41 (88%) cases, among which 21 (58%) were positive for both CK5/6 and EGFR, 14 (39%) were positive for only CK5/6 and 1 (3%) was positive only for EGFR. The remaining 5 cases were negative for both markers (non basal-like carcinoma, NBLC). Overall, TF expression was found in 35 of the 41 (85%) cases. TF expression was detected in 31 of 36 (86%) BLC and in 4 of 5 (80%) NBLC. With respect to EGFR expression, 18 of the 22 (81%) cases positive for EGFR expressed TF, however TF expression was also found in 4 of 6 (80%) cases that lacked EGFR expression.

Conclusions: TF expression was found in the majority of cases of TNBC (88%), suggestive that TF expression is linked with the aggressive tumor behavior and poor prognosis of the patients with TNBC. Tumor TF expression was similar in BC (86%) and NBC (80%). However, a close association between TF and EGFR expression was not observed. Further study is warranted to explore the clinical significance of TF expression and its association with EGFR expression in this breast cancer subtype.

Cardiovascular

351 Causes-Mechanisms of Death Following Stage I Repair for Hypoplastic Left Heart Syndrome (HLHS)

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Background: Staged hybrid repair is a recent advancement in treatment of HLHS. Stage I (of 3 stages) includes balloon atrial septostomy and ductus arteriosus stenting (by catheterization) as well as surgical pulmonary artery banding. The causes-mechanisms of death between the stage I and II procedures have not been well-documented at autopsy.

Design: Autopsy reports and microscopic slides were reviewed from 13 consecutive patients (August 2002 - August 2009) who died after first stage repair for HLHS (6 males and 7 females). Causes-mechanisms of death as well as nonfatal complications were recorded.

Results: The mean age at death was 60 days (range 9 to 180 days). Six deaths occurred at < 30 days following stage I repair (group 1) and 7 deaths occurred at > 30 days (group 2). Eleven patients had the complete stage I repair, one had only atrial septostomy and one had only ductus arteriosus stenting. Autopsy permit included no restrictions (n=7), chest and abdomen only (n=2) or chest only (n=4). The causes-mechanisms of death in group 1 were ductus arteriosus closure (n=1, patient had only atrial septostomy), left atrial appendage tear (n=1, occurred during atrial septostomy), arrhythmias that developed during the procedure (n=3) and pneumonia (n=1). Two of the patients with arrhythmias had a myocardial substrate that may have predisposed to arrhythmia (fibrosis and myocyte disarray). The causes-mechanisms of death in group 2 were intestinal necrosis (n=1), myocardial necrosis due to insufficient retrograde flow into the aortic arch (n=1), pneumonia (n=2), pulmonary emboli (n=1), pulmonary vein stenosis (n=1) and sudden death without documented arrhythmia (n=1). Aorta and/or vena cava thrombosis or a significant thromboembolic event were found in four group 2 patients.

Conclusions: Intraprocedural events (arrhythmia, left atrial tear) accounted for 67% of deaths in group 1 patients. Pneumonia and ischemic necrosis (heart, intestines) accounted for the majority of deaths (57%) in group 2. Thrombotic / thromboembolic