(p25q13) are prominent. The objectives of this study were to: 1) broaden the currently available cytogenetic data on this unusual entity by subjecting additional CMFs to karyotypic analysis; 2) further localize the recurrently involved 6q13 breakpoint; and, 3) uncover potential candidate gene(s).

Design: 15 CMF specimens from 13 patients were analyzed utilizing standard cytogenetic analysis. A fluorescence in situ hybridization (FISH)-based positional cloning strategy on CMF abnormal metaphase cells using a series of bacterial and P1 artificial chromosome (BAC/PAC) probe combinations spanning a 6.1 Mb region was employed for narrowing the 6q13 breakpoint. Following identification of the BAC/PAC probe sets most closely approximating the critical 6q13 breakpoint, additional FISH studies were conducted on interphase cells obtained from cytologic touch preparations of 12 CMFs.

Results: Chromosome 6 abnormalities were detected in 9 of the 10 clonally abnormal CMFs. In addition to 6q13 rearrangements, recurrent 6p25 and 6q25 anomalies were detected. FISH studies demonstrated that the definitive 6q13 breakpoint locus was flanked proximally by a RP11-560O20, RP11-536O4 and RP1-238D15 BAC/PAC probe cocktail and distally by a RP11-209D8 and RP1-234P15 BAC/ PAC probe cocktail; a region encompassing the *COL12A1* gene locus. Abnormalities of 6q13 were identified by metaphase and/or interphase cell FISH analysis in 2 of 14 (14%) CMFs.

Conclusions: These cytogenetic and molecular cytogenetic findings expand our knowledge of chromosomal alterations in CMF, further localize the critically involved 6q13 breakpoint with identification of *COL12A1* as the likely gene candidate, and provide an alternative approach for detecting 6q13 abnormalities in nondividing cells of CMF. The latter could potentially be utilized as an adjunct in diagnostically challenging cases.

Breast

104 Histologic Grading of Invasive Lobular Carcinoma: Does Use of a Two-Tiered Nuclear Grading System Improve Interobserver Variability?
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Background: The Nottingham histologic grade (NHG) is an established prognostic marker for infiltrating ductal carcinoma. Its usefulness in the case of invasive lobular carcinoma (ILC) has been less clear, given that two of the three parameters, tubule formation and mitotic activity, show little variation in ILC, thereby placing much of the emphasis on nuclear grade. We have previously reported a trend for improved overall and relapse-free survival in patients with ILC of low nuclear grade, as classified by a two-tiered nuclear grading system. Given the inherent potential for interobserver variability with any grading system, the goal of the current study is to compare the degree of interobserver variability in the grading of ILC utilizing a two-tiered nuclear grade versus the NHG.

Design: Representative sections from 38 cases of ILC were graded independently by 5 pathologists using NHG criteria for tubule formation, nuclear pleomorphism, and mitotic activity. In addition to the NHG, tumors were categorized by a two-tiered nuclear grading system as low grade (grade 1 nuclei) or high grade (grades 2-3 nuclei). Pair-wise kappa values and interobserver agreement rates were calculated for both the NHG and the nuclear grade, and results were compared using the paired t-test.

Results: Results are summarized in the table. Overall, mean kappa values demonstrated only fair (NHG) to moderate (nuclear grade) agreement. A statistically significant difference was observed between kappa values for NHG compared to those for nuclear grade. Interobserver agreement rates also showed improvement with use of the nuclear grading system as compared to NHG.

	NHG	Nuclear Grade	P Value
Kappa	0.0828 to 0.572 (mean 0.3228)	0.304 to 0.695 (mean 0.4738)	0.0021
Interobserver Agreement Rate	53-79% (mean 70%)	68-92% (mean 83%)	<0.0001

Conclusions: Interobserver variability is to be expected with any histologic grading system. Given that histologic grade has prognostic implications for breast cancer patients which may guide treatment choices, accurate reporting of histologic grade is paramount. In the case of ILC, where use of the traditional NHG places substantial weight on the criterion of nuclear pleomorphism, a two-tiered nuclear grading system may reduce interobserver variability yet still provide useful prognostic information.

105 Molecular Profile of Breast Cancer Metastases to the Central Nervous System
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Background: Breast cancer (BC) is the second most common cause of central nervous system (CNS) metastases, developing in 10-20% of patients with BC. Previous studies have suggested that patients with positive HER2 status BC developed CNS metastases within 16 months, thus, development of new molecular therapies that interfere with HER2 pathways are needed. We studied primary tumors and corresponding CNS metastasis of patients with BC to compare the expression of molecular markers (ER/PR, HER2, EGFR) in both locations and to identify molecular profile of primary BC which could indicate an increased risk for spread to the CNS.

Design: Twenty patients with primary BC and their corresponding CNS metastases were studied. Clinical data and pathologic features were reviewed. Treatment of primary BC included surgery, chemotherapy and radiation. All cases were evaluated for estrogens-(ER)/progesterone-(PR) receptors, HER2 and EGFR expression by immunohistochemistry (IHC). HER2 status was also evaluated by chromogenic in situ hybridization (CISH).

Results: Patients had an average age of 47 years (range 27-70years). All primary tumors were invasive ductal carcinomas, of moderate (35%) and high histologic grade (65%). Primary BC show negative ER/PR, positive EGFR and positive HER2 status in 56%, 31%, and 30% of cases respectively. A 56 % of tumors expressed positive EGFR and/or HER2 status with negative ER/PR. CNS metastases had the same ER/PR, EGFR and HER2 status as the primary tumors in 95%, 80% and 95% of cases respectively. In both primary and metastatic lesions, we found high concordance between IHC and CISH in HER2 status testing. All IHC negative (0/1+) and positive (3+) cases were unamplified and amplified by CISH respectively. IHC-equivocal (2+) results were found in 7/40 (17%) samples, corresponding to 5 primary and 2 metastatic tumors. In these cases, CISH confirmed gene amplification in 2 primary lesions and nonamplification in the remaining five tumors.

Conclusions: Our study suggests that patients affected by primary BC with negative ER/PR and positive HER2 or EGFR status are more susceptible to develop metastases to the CNS. Molecular profile of the primary tumor has been maintained in the CNS metastasis.

106 Can We Predict Which Cases of Atypical Ductal Hyperplasia on Breast Core Needle Biopsy Will Upgrade?
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Background: Prior studies suggest atypical ductal hyperplasia (ADH) involving ≤2 foci at 11- or 14-gauge stereotactic vacuum assisted breast biopsy (VABB) may not require surgical excision because upgrading to carcinoma does not occur.

Design: Retrospective review of 991 consecutive 9- or 11-gauge stereotactic VABB procedures from February 2001 through June 2006 identified 94 cases performed for mammographic calcifications, confirmed to contain ADH on blinded pathology review. All of these cases had subsequent surgical follow-up. Each large duct or terminal duct-lobular unit containing ADH was counted as a focus and the total number of foci were determined for each case. The largest span of contiguous ADH was measured. The presence of a micropapillary growth pattern or findings suspicious for DCIS was noted. Pathology reports of the excisional biopsy specimens were reviewed to determine which cases upgraded.

Results: Fifteen of 94 (16%) cases of ADH upgraded to carcinoma on excision (13 DCIS, 2 invasive). Cases with > 2 foci were significantly more likely to upgrade (12 of 51 upgraded, P=0.045, Fisher's exact test), but the risk of upgrade for cases with ≤ 2 foci was 7% (3/43). The greatest diameter of ADH in the 15 cases that upgraded ranged from 0.2-5.0 mm with 27% measuring ≤ 1.0 mm. The subjective interpretation of "suspicious for DCIS" was a significant predictor of upgrade with 35% (8/23) of cases categorized as suspicious upgrading compared to 10% (7/71) of cases that were not called suspicious (P=0.008). Micropapillary features were noted in 10/15 (67%) cases that upgraded compared to 35/79 (44%) cases that did not upgrade but the result did not reach statistical significance.

Conclusions: The risk of upgrade of ADH is associated with the number of foci involved and subjective suspicion for DCIS. However, upgrade at surgical excision can occur even when ≤ 2 foci of ADH are found at VABB. A standardized method of reporting ADH could be used to assess individual patient risk of upgrade and make recommendations for surgical excision.

107 Florid Lobular Intraepithelial Neoplasia (FL-LIN) with Signet Ring Cells (SRC), Central Necrosis and Calcifications: A Clinicopathologic and Immunohistochemical Analysis of Ten Cases
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Background: In the most florid type of ductal involvement, LIN proliferates to form a solid mass of tumor cells that fill and expand the duct lumen. Foci such as these can develop central necrosis and calcifications, which are detectable on mammograms. The immunohistochemical expression of E-cadherin has been found to be absent in virtually all reported examples of LIN with necrosis. FL-LIN with necrosis may consist of classical or pleomorphic cell types. However, the occurrence of LIN composed entirely of signet ring cells with central necrosis is extraordinarily rare. We describe herein 10 examples of these cases, to illustrate this uncommon morphologic pattern of Lobular Intraepithelial Neoplasia.

Design: The cases were encountered during routine clinical practice of the authors performed over a 5-year period (2002-2007) at Mexican Oncology Hospital. In all cases, we analized the expression of E-cadherin(E), high-molecular-weight keratins (HMWK), Estrogen Receptor (ER), Progesteron Receptor (PR) and Her2/neu. Patient´s clinical information was obtained from the medical records.

Results: We reviewed 10 patients with florid LIN with SRC and central necrosis, patient´s ranged in age from 45 to 75 years (mean:51.2). Clinical profiles of patients were not significant different from those with classical LIN. The main indications for biopsy were calcifications (n:7) and mass (n:3). On mammography, all calcifications were clustered, punctuate, high density and smaller than or equal to 0.6mm. Luminal necrosis was present in all cases and calcifications in 7 of FL-LIN with SRC. Eight patients (80%) had associated invasive carcinoma including 5 classical lobular, and 3 invasive lobular carcinoma classical type with signet ring cells. Immunoreactivity for ER, PR and HMWK was present in 9/10(90%), 8/10(80%) and 9/10(90%) of cases respectively. All cases had complete absence of staining for E. Overexpression of Her 2/neu was absent in all 10 cases.

Conclusions: Lobular Intraepithelial Neoplasia composed entirely of signet ring cells can develop extreme ductal and lobular enlargement, central necrosis and calcifications. These cases are frequently associated with invasive lobular carcinoma.

108 The Incidence of Concurrent Lobular Carcinoma In Situ (LCIS), Columnar Cell Lesions (CCL) and Tubular Carcinoma (TC): An Analysis of 105 Cases
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Background: Recent study (Brandt, et al, Adv in Anat Pathol 2008; 15(3): 140-6) have described a strong association between LCIS, CCL and TC. The "Rosen Triad", named in tribute to its first categorical description by the eponymous pathologist, is a morphological observation that may have important clinical and pathologic implications. There is no appreciable literature that addresses the simultaneous occurrence of these 3 lesions. In this study, our aim was to evaluate the frequency of this "triad" in our patient population.
Design: The archives of the Department of Pathology, Mexican Oncology Hospital were retrospectively searched to identify cases of TC diagnosed from 1999 to 2007. Only excisional biopsies or mastectomies were included. TC was composed of more than 90% ducts that were well formed with a single layer of epithelial cells with low grade nuclei and separated by a desmoplastic stroma. The CCLs were classified into 3 different categories (CCL without hyperplasia, CCLs with hyperplasia lacking atypia, and CCLs with atypia). LCIS was defined as a population of neoplastic cells causing expansion and enlargement of the lobules. We also examined various clinical factors (age, presentation, tumor size). The presence of an associated ductal carcinoma in situ (DCIS) was also assessed. Sections from each case were evaluated by immunohistochemistry with hormonal receptors (ER, PR) and Her 2/neu.
Results: We identified 105 patients with Tubular Carcinoma with a mean age at diagnosis of 55 yr, the mean tumor size was 1.5cm. All patients presented with a radiographically detected mass. None of the patients were found to have multifocal or multicentric tumors. In 63 of 105(60%) cases of TC, both LCIS and at least one type of CCL were identified. All elements of the triad coexisted within the same space. In the triad group, CCLs with atypia were identified in 44 of 63 patients(70%), 22% (14/63) cases were associated with CCLs with hyperplasia without atypia. DCIS was present in 26 of 63(41%) triad cases. All 3 lesions (TC, CCL and LCIS) were ER positive, PR positive and Her-2/neu negative.
Conclusions: Our study of 105 patients shows that TC is often associated with CCL and LCIS (60% of cases). These results are consistent with the study of Brandt et al. These findings support the hypothesis that TC and LCIS have direct evolutionary links to CCLs.

109 Caracterisation of Breast Cancer Diagnosed during Pregnancy or during the Three Years after Delivery by Tissue Array
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Background: Pregnancy associated breast cancer are defined as breast cancer occurring during pregnancy or during the year after delivery. There is a little increase of breast cancer after pregnancy during the five years after delivery. The course of these patients is more aggressive, and these tumors are frequently hormonal receptors negative and HER2 positive. We tried to define the phenotype of these tumors.
Design: 141 breast cancers diagnosed in women aged 43 or younger were retrieved from our files. Clinical course and pregnancy story was known. We analysed tissue array data from paraffin blocks for protein expression of several markers: estrogen and progesterone receptors, prolactine receptor, Her2, Ki 67, CK 5/6, CK 14, CK 18, vimentine, Ckit, EGFR, P Cadherine , E cadherine, bcl2, P53, p63, cox 2, and Muc1.
Results: During pregnancy or the 3 years after delivery, there was an increase of grade 3 tumors (p=0.05), hormonal receptor negative (p=0.009) and HER 2 positive (p=0.018) phenotype. The triple negative phenotype was also increased (p=0.07). The tissue array data confirmed the results of tumor analysis with an increase of receptor negative tumors and HER2 positive tumors. Moreover, bcl2 and prolactin receptor expression were decreased and p53, P63, and vimentin expression increased. Triple negative tumors have basal phenotype with expression of C-kit, EGFR, CK 5/6, and CK 14. RE positive tumors showed expression of Bcl2 and CK18. Non supervised hierarchical cluster analysis revealed, as previously described, 3 distinct subtypes : basal, her2 positive, and luminal.
Conclusions: Non supervised hierarchical cluster analysis showed that tumors diagnosed during 3 years after delivery are mainly of basal and HER2 positive phenotype. The presence of these phenotypes can be important as concerns systemic treatment issues.

110 Cofilin Expression in Breast Carcinoma with Hormone Receptor-Positive: Prognostic Significance
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Background: Cofilin is a protein required to cellular motility and regulation of actin polymerization. The cofilin pathway has been implicated in tumor cell invasion and metastasis. Overexpression has been demonstrated in cell lines of lung, pancreatic, glioblastoma and breast tumors. However, several studies have found cofilin downregulated in hepatocellular carcinoma (HCC) cells with high metastatic potential. The aim of the current study was to investigate the expression and the prognostic significance of cofilin in a series of breast carcinoma (BC) with positive hormone receptor (HR) status.
Design: A total of 177 cases of BC with HR-positive/Her2-negative with lymphadenectomy and without neoadjuvant treatment were retrieved. Median clinical follow-up was 55 months (range 11 to 165 months). Age ranged from 42 to 88 years (median 61 years). Histologic grade (HG) was assessed according to the Nottingham criteria. Immunohistochemical (IHC) staining was performed in whole sections for Bcl2 (cut-off 50%), ER (cut-off 10%), PgR (cut-off 10%), Ki67 (cut-off 20%), p53 (cut-off 20%) and Her2 (2+ and <30% 3+ confirmed by FISH). Further IHC for cofilin (cut-off

score 50; range 0-300), CK5/6 (cut-off 10%) and EGFR (cut-off 10%) was performed on tissue microarrays. Significant associations were identified using Chi-square and Fisher's exact test. Actuarial survival was calculated by the Kaplan-Meier method (log rank test). A p-value <0.05 was considered significant.
Results: Increased cofilin expression was observed in 31% of tumors, low Bcl2 in 27%, high Ki67 in 21%; CK5/6 was positive in 12%, p53 in 4% and EGFR in 0,6%. Tumors with cofilin overexpression showed lower HG (p=0.03) and positivity for Bcl2 as a trend (p=0.06). Poorer overall survival was seen for patients with larger tumors (>20 mm) (p=0.03), of G3 (p=0.05), positive lymph nodes (p=0.02) and low Bcl2 (p=0.01). In contrast, cofilin overexpression was strongly associated with a better outcome (100% versus 87 % in negative cases; p=0.0007).
Conclusions: Our findings in a series of patients with BC and HR-positive status show that cofilin expression is associated with good prognostic factors, such as low grade tumors and Bcl2 overexpression as well as with longer patients' survival. Therefore, cofilin is a favourable prognostic factor, in contrast to previous reported data in cellular assays. Supported by grant FIS PI061488.

111 Automatic Detection of Micrometastases in Lymph Nodes by Infrared Micro-Spectral Imaging
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Background: Infrared micro-spectral imaging (IRMSI) is a novel, optical technique that can provide a rapid measurement of sample biochemistry and identify variations that occur between healthy and abnormal tissue. The advantage of this method is that it is objective and provides reproducible results, independent of fatigue, experience and inter-observer variability. This study explores the correlation between the spectral and conventional histopathology of axillary lymph nodes containing breast micrometastases (MMT).
Design: Twenty axillary lymph nodes, known to have contained breast MMT's, were selected from the Tufts Medical Center tissue archive. Unstained tissue sections were cut for investigation using IRMSI. The tissue section was interrogated by a beam of IR light that sampled pixels (6.25 µm x 6.25 µm in size) and consecutive 1 mm x 1 mm IR images were recorded from regions that previously showed MMT's. Each IR image consisted of 25,600 complete IR spectra that describe the tissue's discrete biochemistry at each pixel coordinate. By use of Hierarchical Cluster Analysis (HCA), IR spectra were sorted into groups dependent upon their spectral similarity and pseudo-color images were constructed to compare against conventional histopathology following H&E staining of the tissue. Correlating the tissue's spectral and histopathological features, a diagnostic computer algorithm was generated.
Results: More than 30 IR images from axillary lymph nodes were recorded for a total of 750,000 IR spectra. Multivariate analyses of the data revealed that each tissue type displayed its own distinct spectral pattern. Adipose tissue showed prominent IR bands associated with lipids, whereas the lymph node capsule showed strong IR bands associated with collagen. Spectral differences between lymphocytes and MMT's were also readily discernible, with distinct variations occurring to both the protein and phosphate composition of abnormal tissue. More importantly, small micrometastases (<150µm), as well as small clusters of individual cancer cells (<25µm) were correctly identified using this technique.
Conclusions: IR micro-spectral imaging can identify small micrometastases within excised tissue. The utilization of optimized IR imaging instruments may allow the acquisition and diagnosis of entire lymph nodes within a few minutes.

112 Can Factors Evaluated in the Routine Pathologic Evaluation of Lymph Node-Negative Estrogen Receptor-Positive Stage I or II Invasive Breast Cancer Be Used To Predict the Oncotype DX Assay Recurrence Score?
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Background: Oncotype Dx is a commercially available multigene RT-PCR assay which is used to quantify the risk of breast cancer recurrence in patients with stage I or II estrogen receptor-positive lymph node (LN) negative invasive breast cancer who will be treated with tamoxifen. Patients with high recurrence score (RS) derive a large benefit from cytotoxic chemotherapy while those with a low RS derive little if any benefit from chemotherapy. This study is to determine if progesterone receptor (PR) evaluation or mitotic count score, which are part of the routine pathologic evaluation of all breast cancers, could predict Oncotype Dx assay RS results.
Design: Thirty-seven cases of LN negative, ER positive invasive breast cancer were identified where both results of an Oncotype Dx assay and slides were available for review. In all cases, the slide from the block sent for Oncotype Dx was reviewed and evaluated using the Nottingham grading system. ER, PR and Her2/Neu status was available. The Oncotype RS was obtained with the number and category of risk as follows: <18 (low), 18-30 (intermediate) and >30 (high).
Results: Of the 37 patients with BC in the study, 22 were low, 13 were intermediate and 2 were high risk for BC recurrence according to the RS guidelines. All cases were ER positive. There were 7 PR negative cases and in these cases the RS was 18, 20, 20, 23, 30, 34 and 40 respectively. There were no low risk RS among the PR negative cases. Thirty patients were PR positive and of those 23 had a RS of 17 or less. There were only 5 cases with a mitotic count score of 3 and in these cases the RS was 16,20,23,34 and 49 respectively. In 2 cases with RS of 34 and 49, mitotic count score was 3 and PR was negative. In 4 patients with a RS of greater than 17 both PR was positive and the mitotic count score was less than 3.
Conclusions: In our study, breast cancer cases with both a mitotic count score less than 3 and PR positivity, we could not accurately predict RS. However, cases with an invasive breast cancer showing absence of progesterone receptor and/or mitotic count of 3 can be predicted to have an intermediate or high RS. If the goal of the oncologist

is to omit chemotherapy, the contribution of RS determination in this subset of patients (PR negative and/or mitotic count score of 3) may be low.

113 Universally-Primed Quantitative Multiplex PCR Short Fluorescent Fragments (upQMPSF): A New Detection Method for *HER2* Amplification in Breast Cancer
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Background: Amplification of *HER2* in breast cancer identifies patients eligible for Trastuzumab therapy. HER2 status can be evaluated either by immunohistochemistry (IHC) or by fluorescent in situ hybridization (FISH). Interobserver variability is still a problem. The purpose of the study is to develop a new PCR based technology that is more accurate, reliable, less subjective, cost-effective and easier to interpret.

Design: universally-primed Quantitative Multiplex PCR Short Fluorescent Fragments (upQMPSF) for the detection of HER2 amplification involves a multiplex set consisting of 7 genomic regions. These include 4 exons of *HER2*, and 3 other regions that include 2 flanking the centromere of chr17 and one outside chr17 as internal controls. This approach identifies chr17 polysomy. Compatible multiplex primers were designed to cover the entire exon regions. Furthermore, the numbers of PCR cycles were empirically optimized to retain the quantitative capacity of the PCR, and the products were analyzed using the ABI capillary sequencer. Sixteen random samples of breast cancer that had tumor and normal frozen tissues were analyzed using FISH and upQMPSF. For upQMPSF method, DNA was extracted and for FISH analysis, PathVysion method was used. HER2 was positive by FISH when *HER2:* CEP17 ratio was more than 2.2 and negative when this ratio was less than1.8. HER2 was positive by upQMPSF when the ratio of overall average for *HER2* peak heights for all exons over the internal control in tumor versus normal was ≥ 50%. There was no equivocal result.

Results: HER2 FISH identified 4 (25%) positive cases and 12 (75%) negative. upQMPSF identified 3 (18.8%) positive cases and 13 (71.2%) negative. The agreement between FISH and upQMPSF was in 15 of 16 (93.7%) with confidence interval (0.7-1.0). upQMPSF requires 200 nanogram DNA from tumor and normal tissues to run the test. The test takes 1 hour after DNA extraction to get final result.

Conclusions: upQMPSF method may offer an alternative method for the detection of *HER2* amplification and may be particularly useful in improving the accuracy of cases that have equivocal results (FISH 1.8-2.2). Given the small amount of DNA required, the test has advantage over other tests like FISH and IHC when the tested tumor is small. A larger study will be conducted to validate these results.
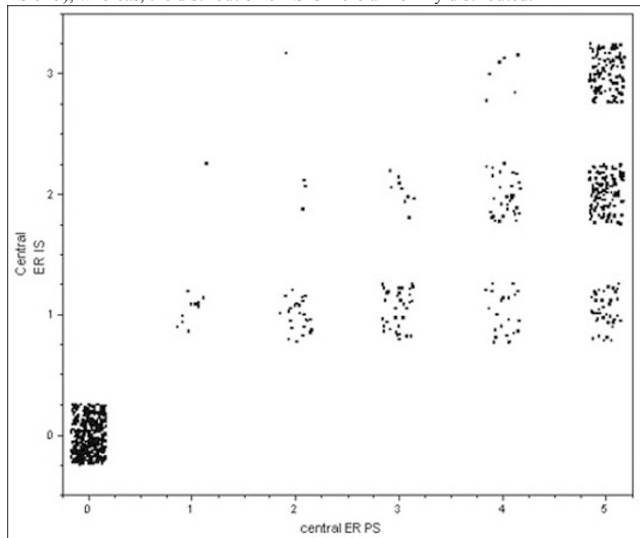
114 ER and PR Assessment by Central IHC: Examination and Comparison of Percent Positive Cells and Nuclear Staining Intensity in ECOG Breast Cancer Study 2197
S Badve, R Gray, B Childs, T Maddala, M Liu, S Rowley, N Davidson, S Shak, LJ Goldstein, GW Sledge, JA Sparano, FL Baehner. ECOG, Indianapolis, IN; Sanofi-aventis, Bridgetown, NJ; Genomic Health, Redwood City, CA; UCSF, San Francisco, CA.

Background: Accurate laboratory assessment of hormone receptors (HR) in breast carcinoma is therapeutically important. Using central laboratory IHC results for ER and PR, we explored the Allred Score and its constitutive components, the nuclear intensity score (IS) and proportion score (PS), and examined these measurements to patient outcome data.

Design: Tumors from 776 pts (179 of whom relapsed) enrolled on E2197 were examined; pts had 0-3 positive nodes and all received doxorubicin and cyclophosphamide or docetaxel plus hormonal therapy (if local laboratory HR+). Central IHC for ER (1D5) and PR (636) used two 1.0 mm tissue microarrays. The staining intensity (0-3 scale) and proportion of positive cells (0-5 scale) were reported and the Allred score (AS) that combines the two was calculated.

Results: The Spearman rank correlation between ER IS and PS was high (0.98). The same high correlation was observed for PR IS and PS. For ER, the distribution of proportion score tended to be bimodal (45% of patients have a PS of 0 and 36% of patients have a PS of 5); whereas, the distribution of IS is more uniformly distributed.

These distributions are reflected in the Allred score which also tended towards a bimodal distribution (58% of ER positive patients have an Allred score of 7 or 8). ER IS and ER PS are both significantly associated with recurrence (p=0.0006 and p<0.0001 respectively). While the 5-year recurrence rates decreased monotonically from 15% in PS category 2 to 7% in PS category 5, the 5-year recurrence rates for IS decreased from IS categories 0 to 2 (from 16% to 7%) but then increased again for category 3 (10%). The pattern of 5-year recurrence rates by IS category was similar to that for the Allred Score.

Conclusions: By central IHC assessment of ER and PR status using the Allred Score, there is a high degree of correlation between the intensity score and proportion score, and both are significantly associated with 5-year recurrence rates.

115 HER2 Concordance between Central Laboratory Immunohistochemistry, FISH and Quantitative RT-PCR in Intergroup Trial E2197
FL Baehner, R Gray, B Childs, T Maddala, S Rowley, S Shak, NE Davidson, GW Sledge, LJ Goldstein, JA Sparano, S Badve. UCSF, San Francisco, CA; ECOG, Indianapolis, IN; Sanofi-aventis, Bridgewater, NJ; Genomic Health, Redwood City, CA.

Background: Laboratory assessment of HER2 is of marked clinical importance. Accurate quantification remains problematic within and between laboratories. Here we report central laboratory HER2 results comparing IHC, FISH and quantitative RT-PCR using Oncotype DX in patients enrolled in a large adjuvant breast cancer trial.

Design: 755 patients with 0 to 3 positive lymph nodes from Intergroup study E2197 were studied. Central IHC for HER2 was performed using duplicate 1.0 mm microarrays (HercepTest™; Dako). Percent positive cells and staining intensity (0-3+) was assessed, where IHC positive cases exhibited 3+ staining in >30% cells, IHC equivocal cases exhibited 3+ staining in <30% cells or 2+ staining, and IHC negative cases exhibited 0 or 1+ staining. For HER2 measurement by FISH, analysis for amplification ratio is in progress by a central laboratory with positive >2.2, equivocal 1.8 to 2.2, and negative < 1.8. Quantitative RT-PCR for HER2 used Onc*otype* DX and pre-defined cutoffs: positive ≥11.5 units, equivocal >10.7-<11.5 units, and negative ≤10.7 units (each unit represents a 2-fold change in expression). Concordance analysis excluded the equivocal range from both assays according to ASCO/CAP Guidelines (Wolff et al, 2006).

Results: HER2 expression by IHC and by RT-PCR is shown in the Table below. Of the 134 cases positive by IHC, 27 (20%) cases were negative by RT-PCR. 175 cases were equivocal by IHC (165 of these were HER2 negative by RT-PCR). 26 cases were equivocal by RT-PCR (3 of these were HER2 negative by IHC). The overall concordance for HER2 status by central IHC and central RT-PCR was 95% (95% CI, 92%, 96%). FISH hybridizations are complete, analyses are in progress and results will be presented at meeting.

HER2 Concordance: RT-PCR & IHC

HER2	Central IHC+	Central IHC equivocal	Central IHC-	Total RT-PCR
RT-PCR+	94	0	4	98
RT-PCR equivocal	13	10	3	26
RT-PCR-	27	165	439	631
Total IHC	134	175	446	755

Conclusions: There is a high degree of overall concordance between central IHC and central RT-PCR positive and negative HER2 cases. Assessment of HER2 status by RT-PCR using Oncotype DX is an alternative to IHC.
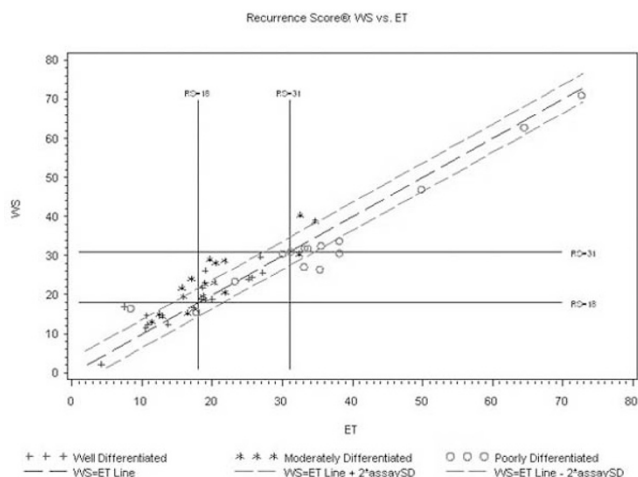
116 Biopsy Cavities in Breast Cancer Specimens: Impact on Quantitative RT-PCR Gene Expression Profiles and Recurrence Risk Assessment
FL Baehner, C Quale, C Pomeroy, D Cherbavaz, S Shak. UCSF, San Francisco, CA; Genomic Health, Inc, Redwood City, CA.

Background: The 21-gene recurrence score assay is prognostically and predictively important in ER+ breast cancer. Manual microdissection is performed in cases where biopsy cavities (BxC) are present. The objective of this study was to characterize by quantitative RT-PCR the impact of BxC on 21 gene expression profiles and the Recurrence Score (RS).

Design: 48 (15 well, 18 moderate, and 15 poorly differentiated) invasive breast carcinomas were evaluated for differences in gene expression between whole sections (WS; which contained BxC) and enriched tumor (ET; where BxC were excluded by manual microdissection). Standardized quantitative RT-PCR analysis for the 21 genes was performed; reference normalized gene expression measurements ranged from 0 to 15, where each 1-unit reflects an approximate 2-fold change in RNA. Analyses of individual genes and RS were performed on the entire sample set and stratified by tumor grade. Correlation analyses used Pearson's R, concordance analysis by Lin's sample concordance and paired t-tests to characterize differences.

Results: Of the 16 cancer-related genes there were statistically significant differences in reference normalized gene expression between ET and WS in 6 genes: BAG1 (ET-WS: 0.13 units, p=0.0025), CD68 (ET-WS: -0.64 units, p<0.0001), ER (ET-WS: 0.29 units, p=0.0012), GSTM1 (ET-WS: 0.18 units p=0.0025), STK15 (ET-WS: -0.18 units, p=0.0041) and STMY3 (ET-WS: 0.62 units, p<0.0001). Expression of CD68 was higher and ER was lower in WS containing BxC. The correlation (0.95) and concordance (0.92) were generally high between WS and ET for RS overall; however, among moderately differentially tumors, there was a statistically significant mean increase in RS for WS of 3.3 units (p = 0.0012) while among poorly differentiated tumors, there was a trend toward a statistically significant decrease in RS for WS of 2.2 units (p=0.0569).

Recurrence Score® WS vs. ET

+ + + Well Differentiated
— — WS=ET Line
∗ ∗ ∗ Moderately Differentiated
— — WS=ET Line + 2*assaySD
○ ○ ○ Poorly Differentiated
— — WS=ET Line - 2*assaySD

Conclusions: The inclusion of BxC in breast cancer specimens is associated with significant changes in the expression of individual genes and can impact the RS. The removal of BxC by manual microdissection from breast cancer specimens assessed for gene expression levels is warranted.

117 Expression of Potential Cancer Stem Cell Markers CD44+/CD24- in the Molecular and Genetic Subgroups of Breast Cancer
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Background: In the stem cell model of carcinogenesis it is the small population of cancer stem cells (CSC) in a tumor that is believed to be the driver of tumorigenesis, the source of metastasis and the cause of relapse and therapy resistance. In breast cancer a population of cells identified as CD44+/CD24- have been demonstrated to have CSC properties *in vitro* and *in vivo*. Breast cancer is a highly heterogenous disease at the clinical and molecular level, with distinct molecular subtypes strongly correlated with clinical outcome. We hypothesize that a correlation exists between the expression of CD44+/CD24- representing CSCs and the adverse molecular subtypes of breast cancer and with tumor parameters known to portend a poor prognosis.
Design: Tissue microarrays (TMAs) were constructed using 58 *BRCA1*-associated, 64 *BRCA2*-associated and 242 non-BRCA1/2 breast tumors. Immunohistochemical profiling of all tumors was performed with antibodies against ER, PR, CK8/18, CK5, CK14 and EGFR to determine the molecular subtype and CD44 and CD24 to determine if CSCs were present. Statistical comparison was made using the Chi-Square test or Fisher's Exact test.
Results: 81/364 (22%) of tumors were basal-like, 32/364 (9%) were HER2/neu overexpressing, 218/364 (60%) were luminal and the remaining 32 (9%) were unclassifiable. Expression of CD44+/CD24- was positively correlated with the basal-like subgroup (p=0.04); 27% of basal-like tumors, 3.4% of HER2/neu overexpressing and 13% of luminal tumors contained CD44+/CD24- CSCs. Furthermore expression of CD44+/CD24- was positively correlated with adverse prognosis features including high tumor grade (p=0.002), lymphatic invasion (p=0.01) and lymph node positivity (p=0.01). *BRCA1*-associated tumors are known to be enriched for basal-like tumors, and when we examined this subgroup we found expression of CD44+/CD24- was positively associated with *BRCA1*-associated tumors (p=0.01); 28% of *BRCA1*-associated tumors contained CD44+/CD24- cells compared to 13% of non-BRCA1 tumors.
Conclusions: This study demonstrates that basal-like and *BRCA1*-associated tumors are more likely to express CD44+/CD24- than non-basal and non-*BRCA1*-associated tumors. Furthermore expression of CD44+/CD24- correlates with poor histopathologic and/or prognostic characteristics.

118 Estrogen Receptor Expression in Atypical Hyperplasia and Its Association with Type of Atypia and Age
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Background: Estrogen receptor (ER) expression is present in normal breast epithelium and premalignant breast lesions. Prior studies have shown that ER expression increases with age in normal breast epithelium; whereas no age association was seen in atypical hyperplasia and carcinoma in situ.
Design: ER expression was assessed immunohistochemically in archival sections from 246 women with atypical hyperplasia who had an open benign breast biopsy between 1967 and 1991. The ACIS®III (Dako, Carpinteria, CA) was utilized to calculate ER expression (percent staining and staining intensity) in all atypical foci for each woman. Using multivariate linear regression, we examined associations of ER expression with age at biopsy, year of biopsy, indication for biopsy, type of atypia, number of atypical foci, involution status, and family history. Heterogeneity of breast cancer risk across levels of ER expression was also assessed, standardized to a control population (the Iowa SEER registry).
Results: Among the 246 women, 87 (35%) had ADH, 141 (57%) had ALH, and the remaining 18 (7%) had both ADH and ALH. About half (53%) were older than 55 years at diagnosis. Forty-nine (20%) developed breast cancer during a median follow up of 14.4 years. Multivariate analysis indicated that type of atypia, year of biopsy,

and age at diagnosis were significant predictors of ER percent staining and intensity [P<0.05 (see Table 1)]. ER expression was increased in women with ADH and/or those over the age of 55. The relationship between ER (percent staining and intensity) and breast cancer risk in patients diagnosed with atypia was not significant (P=0.099 and P=0.118, respectively).

Multivariate Linear Regression Analysis for Predicting ER Mean Percent Staining and Intensity in Atypical Hyperplasia

Variable	ER Mean Percent Staining (P-value)	ER Mean Intensity (P-value)
Age	0.011	0.008
Year of Biopsy	0.002	0.001
Indication for Biopsy	0.706	0.868
Type of Atypia	<0.001	<0.001
Number of Foci	0.584	0.374
Involution	0.277	0.378
Family History	0.899	0.993

Conclusions: This study suggests that ER expression is higher in ADH. Significance of year of biopsy could be due to age of tissue and its effect on immunohistochemistry. Contrary to previous reports, our results indicate that ER expression in atypical hyperplasia increases with age.

119 Mammary Intraductal Foam Cells Are Bone Marrow Derived and Are Recruited in Response to Both Physiological as Well as Neoplastic Stimuli
SH Barsky, Y Xiao, Y Ye, K Yearsley. The Ohio State University College of Medicine, Columbus, OH.
Background: Intraductal "foam cells" are the most commonly encountered cells in spontaneous nipple discharge, nipple aspirate fluid and ductal lavage yet their origin and significance remain a mystery. These cells increase in pregnancy and other conditions of ductal ectasia and obstruction. They frequently surround DCIS. These foam cells often ingest both endogenous as well as exogenous substances. Because these macrophages were observed intraductally and because their appearance resembled lactating, vacuolated epithelial cells, their origin had been assumed to be of ductal lining epithelium. Previous studies by us and others with macrophage (CD68, lysozyme), epithelial (cytokeratin, estrogen receptor) and myoepithelial (smooth muscle actin, CALLA, maspin) markers suggested that foam cells were of macrophage lineage and terminally differentiated (negative Ki-67 and PCNA). Because these observational IHC findings suggested a possible bone marrow-derived monocyte origin, we decide to conduct experimental studies to prove this hypothesis.
Design: We conducted two types of murine bone marrow transplant studies: Donor marrow from female GFP-transgenic C57 black mice were transplanted into sublethally irradiated female C57 recipients rendered pseudopregnant with a combination of estradiol, progesterone and estriol (2.5mg) 21 day release pellets. Donor marrrow from female ROSA26 containing the lacZ reporter were transplanted into irradiated female recipient transgenic mice carrying potent breast cancer oncogenes: the *MMTV-pymT* and the *MMTV-erbb2/neu* which result in breast cancer. Some of the transgenic recipients were also rendered pseudopregnant.
Results: In all of the transplanted recipient mice, the intraductal foam cells expressed the donor marker, either GFP or lacZ. However the number of donor-derived intraductal foam cells were increased in pseudopregnancy 10 fold, by intraductal neoplasia 5 fold and by a combination of the two over 25 fold. Ducts containing neoplastic cells with the highest numbers of mammary foam cells exhibited a significantly increased apoptotic index of the neoplastic cells by TUNEL.
Conclusions: The evidence of a bone marrow origin of mammary foam cells suggests a new strategy of delivering therapeutic genes to DCIS, other precancerous lesions or high risk ductal epithelium. This strategy would exploit the omnipresent mammary foam cell, its bone marrow origin and its chemoattraction to the breast in response to both physiological as well as neoplastic stimuli.

120 Significance of c-kit Expression in Breast Ductal Carcinoma
D Bautista, ML Bernal, A Jimenez, I Arranz, S Luna. Hospital Carlos Haya, Malaga, Spain; DG Innovacion Sanitaria, Sistemas y Tecnologias, Sevilla, Spain.
Background: The c-kit gene encodes a transmembrane tyrosine kinase growth factor receptor (CD117) involved in certain neoplasms. Conflicting results concerning c-kit expression have been reported in malignant breast lesions. Using immunohistochemical approaches, c-kit expression is lost in most breast neoplasms. However, an increase of c-kit expression has been associated with aggressiveness and poor outcome. Flt3 (CD135), a tyrosine kinase receptor expressed in normal breast epithelial cells, has been found to interact with c-kit by heterodimerization. This study has tried to characterize c-kit expression changes within the spectrum of breast ductal carcinoma.
Design: One hundred and twenty eight cases of invasive ductal carcinoma were selected from our local Tumor Bank. Expression of c-kit gene was analyzed by quantitative real-time PCR using cytokeratin 18 as expression reference for breast epithelial cells in both, normal and tumor samples. In parallel, c-kit protein expression was evaluated by immunohistochemistry using the polyclonal rabbit anti-human CD117 antibody from Dako and the Bond Polymer Refine Detection from Leica Microsystems. The same method was used to analyze Flt3 protein expression in normal and tumor breast samples using a polyclonal rabbit anti-human antibody from Abcam.
Results: c-kit was expressed at both mRNA and protein levels in normal breast epithelial cells. However, the analysis of tumor regions with different methodologies gave somewhat discordant results. Protein expression was lost in most invasive breast cancers (89,84%) and only maintained in a subset of tumors with high proliferative activity at advance stage (>2cm). mRNA expression was maintained at normal levels in many neoplasms (51,72%) and no relation was found with protein expression. Surprisingly, a decrease of gene expression was statistically associated with large tumor size. We found Flt3 to be expressed in most in situ and infiltrative breast cancers, and no related with c-kit status.

Conclusions: c-kit plays an important role in the maintenance of normal mammary epithelium and neoplastic transformation leads to its lost. The significance of c-kit protein expression in a minority of advanced, highly proliferative breast ductal carcinomas keeps still unexplained since no relation with gene activation or interaction with Flt3 have been found.

121   Expression of Phospho(p)STAT3 and Phospho(p)STAT5 in Breast Cancer and Correlation with Plasma Prolactin Levels
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Background: Signal Transducer and Activator of Transcription (STAT) 3 and 5 are transcription factors activated by multiple ligands. In the breast, in response to prolactin, STAT5 promotes proliferation and milk production. STAT3, which is activated by cytokines such as LIF, promotes apoptosis and involution. We examined expression of pSTAT 3 and 5 in breast cancer and in relation to plasma prolactin levels.
Design: Tissue microarrays (TMAs) were constructed from 3,093 breast cancers from women enrolled in the Nurses Health Study. Of these, 443 patients had matching plasma prolactin levels drawn at diagnosis. Immunohistochemical studies with pSTAT3 and 5 were performed on the TMAs. Tissue cores were scored from 0-3 based on intensity and quantity of nuclei staining. Relationships between expression of pSTAT3 or pSTAT5 and clinicopathologic features were examined.
Results: Interpretable tissue cores were available for 309 subjects. Of these, 195 (63%) and 114 tumors (37%) showed negative/weak (0-1) and moderate/strong staining (2-3) respectively for pSTAT3. 178 (58%) and 131 (42%) tumors showed negative/weak and moderate/strong staining respectively for pSTAT5. Thirty four (11%) tumors were in-situ and 275 (89%) tumors were invasive carcinomas. Absence of expression of pSTAT3 was significantly more common in high grade tumors (p=0.04) and in invasive vs. in situ carcinomas (p=0.04). Though not statistically significant, pSTAT3- status also was associated with lymph node involvement and larger tumor size. Among post menopausal women, there was a significant increase in risk of pSTAT3+ and pSTAT5+ breast cancers in women in the top vs. bottom quartile of plasma prolactin levels (RR= 2.0 [95%CI 1.2-3.2] and 2.3 [95%CI 1.3-4.1] respectively). No association was observed between prolactin levels and pSTAT3- or pSTAT5- tumors.
Conclusions: The transcription factor STAT3, which plays a role in cell death and involution of lactating lobules, is activated in breast cancer. Absence of pSTAT3 expression correlates significantly with invasive cancer and higher tumor grade. Our findings suggest that the role of STAT3 in breast cancer mirrors its physiologic role. An association between plasma prolactin and pSTAT3+ and pSTAT5+ tumors is also seen. However, further studies are needed to fully elucidate the interactions of the STATs on breast cancer pathogenesis.

122   Breast Cancer Molecular Class ERBB2: Preponderance of Tumors with Apocrine Differentiation and Expression of Basal Phenotype Markers CK5, CK5/6 and EGFR
R Bhargava, JM Striebel, DJ Dabbs. Magee-Womens Hospital of UPMC, Pittsburgh, PA.
Background: This is a study of 205 consecutive invasive breast carcinomas (IBCs) to identify the prevalence of molecular subtypes using immunohistologic (IHC) surrogate markers. The principal aim was to identify morphologic features of tumors that belong to the molecular class ERBB2.
Design: The IBCs were classified using IHC surrogate markers-estrogen receptors (ER), progesterone receptors (PR) and HER2. ER and PR were scored using a semi-quantitative H-score like method with a dynamic range of 0-300. HER2 was considered positive only if 3+ by IHC or unequivocally amplified by FISH. The tumors were classified as follows: Luminal A (**LUMA**; ER score 200 or higher, HER2 negative), Luminal B (**LUMB**; ER score 11-199 or PR >10, HER2 negative), Triple Negative (**TN**; ER and PR score 10 or less, HER2 negative), **ERBB2** (ER and PR score 10 or less, HER2 positive), Luminal A-HER2 Hybrid (**LAHH**; ER score 200 or higher, HER2 positive), Luminal B-HER2 Hybrid (**LBHH**; ER score 11-199 or PR >10, HER2 positive). Blocks from 197 cases were available to construct tissue microarrays (TMAs). TMAs were stained with CK5 (clone XM26). Whole tissue sections of ERBB2 tumors were stained with CK5/6 and EGFR. EGFR was scored using criterion similar to HercepTest.
Results:

Molecular Class	Prevalence	Apocrine Differentiation	CK5+
LUMA	55% (n=113)	4/113 (4%)	0/107 (0%)
LUMB	17% (n=34)	2/34 (6%)	4*/30 (13%)
ERBB2	4% (n=8)	7/8 (88%)	5/8 (63%)
TN	15% (n=32)	9/32 (28%)	22/31 (71%)
LAHH	5% (n=10)	2/10 (20%)	0/10 (0%)
LBHH	4% (n=8)	2/8 (25%)	0/8 (0%)

*basal-like morphology in 3 cases
Additional findings in ERBB2 tumors: Majority were high grade, with an average Nottingham score of 8. Moderate lymphoid infiltrate was seen in 5 of 8 (63%) cases and necrosis in 3 of 8 (38%) cases. The tumor cells were + for CK5/6 in 4/8 cases (50%), and showed EGFR 2+ or 3+ score in 5 cases (63%).
Conclusions: Tumors with apocrine differentiation are most often of ERBB2 type. ERBB2 tumors demonstrate some features classically ascribed to TN-basal-like tumors. EGFR overexpression in ERBB2 tumors may have predictive value (use of dual EGFR/HER2 tyrosine kinase inhibitors like lapatinib). The association between ERBB2 phenotype and apocrine morphology also likely explains the long standing enigma in breast pathology, i.e. coexistence of HER2 positive ductal carcinoma in situ (often with apocrine differentiation) and HER2 negative invasive carcinoma (without apocrine differentiation).

123   Concordance between Semi-Quantitative Immunohistochemical Assay and onco*type*DX™ RT-PCR Assay for Estrogen and Progesterone Receptors
R Bhargava, S Beriwal, DJ Dabbs. Magee-Womens Hospital of UPMC, Pittsburgh, PA.
Background: The 21 gene reverse transcriptase polymerase chain reaction (RT-PCR) assay, commercially available as onco*type*DX™ (Genomic Health Inc., Redwood City, CA) is increasingly used in clinical decision making for estrogen receptor (ER)+, lymph node negative breast cancer patients. The company has recently initiated separate reporting of ER and progesterone receptor (PR) expression units (compared to reference genes).
Design: As part of our quality assurance measures, we compared the ER and PR RT-PCR results with the semi-quantitative immunohistochemistry (IHC) on 80 patients. The ER and PR RT-PCR results (performed on resection specimens) were obtained from Genomic Health reports. The RT-PCR test classifies a tumor as ER or PR positive if the expression units are ≥6.5 or ≥5.5 respectively. ER (clone SP1) and PR (clone 1E2) IHC was performed on optimally fixed core breast biopsy samples and semi-quantitation was performed using a modified H-score method which takes into account both intensity and proportion of cellular staining and the score ranges from 0-300. Any nuclear staining by IHC was considered positive. The results of RT-PCR versus IHC were compared qualitatively (positive/negative) and quantitatively using Spearman rank correlation.
Results: Since onco*type*DX™ is currently performed only on ER+ tumors, all 80 tumors in this study were positive for ER by IHC. All these tumors were also identified as ER+ by RT-PCR. Of the 80 cases, 70 were positive and 5 were negative for PR by both tests. The remainder 5 cases were positive by IHC, but negative by RT-PCR. These cases showed weak PR immunoreactivity (mean H-score of 5.8 and range of 2-15). A linear correlation was obtained between RT-PCR and IHC results for both ER (RT-PCR units range: 7.1-12; H-score range: 14-300; correlation coefficient of 0.509) and PR (RT-PCR units range: 3.2-10; H-score range: 0-300; correlation coefficient of 0.664).
Conclusions: The study demonstrated a high degree of concordance between RT-PCR and IHC for both ER (100%) and PR (94%). The study also demonstrates concordance of hormone receptor (HR) results between core biopsy and resection specimens. There was good linear correlation between IHC semiquantitative results and receptor expression levels determined by RT-PCR. The added advantage of IHC is preservation of morphology and accurate assessment of invasive tumors that are admixed with abundant in-situ carcinoma or normal breast tissue. Moreover, IHC can be performed on the same day of biopsy interpretation.

124   Are Luminal A Breast Tumors as Good as They Sound?
R Bhat, FU Garcia, S Soundararajan. Drexel University College of Medicine, Philadelphia, PA.
Background: Among the molecular subtypes of invasive breast carcinomas (BCa), luminal A subtype (ER positive, Her2/neu negative) tumors are associated with a good prognosis, while luminal B (ER positive, Her2/neu positive) tumors are associated with an intermediate prognosis. **Aim:** To compare the Nottingham Prognostic Index (NPI) in luminal type A and B BCa and determine the role of positive Ki-67 (>20%) and p53 (>20%) in these subtypes.
Design: A total of 223 BCa (187 luminal A (ER+, and/or PR+, HER2-); 36 luminal B (ER+, and/or PR+, HER2+) out of 1094 cases from DUCOM Pathology from 1997-2008, with nodal status information and prognostic panel performed and read by image analysis, were included in the study. These were further classified into prognostic groups using the NPI (<3.4, 3.4 – 5.4, and >5.4). Values of Ki67 and p53 for each prognostic group as well NPI component were evaluated. Statistical analysis was done with Student's t test (p<0.05).
Results:

Ki67 and p53 in Prognostic Subgroups of Luminal A and Luminal B Breast Carcinomas

	NPI < 3.4	NPI < 3.4	NPI 3.4 -5.4	NPI 3.4 - 5.4	NPI > 5.4	NPI > 5.4
Number of cases (%)	Luminal A N= 69 (36.8%)	Luminal B N= 7 (19.4%)	Luminal A N= 82 (43.8%)	Luminal B N=15 (41.6%)	Luminal A N= 36 (19.3%)	Luminal B N= 14 (38.8%)
NPI (Mean±S.D)	2.71±0.49	2.87±0.68	4.106±0.53	4.64±0.32**	6.26±0.82	6.53±0.75
Ki67% (Mean±S.D)	12.53±12.2	31.8±19.6*	18.67±12.44	29.4±15.4*	26.59±21.3	27.5±14.1
p53% (Mean±S.D)	5.29±12.9	25.79±32.5	5.44±11.89	20.5±25.7*	4.99±13.96	14.4±19.7

student's t test, **p<0.001; *p<0.05 (Luminal A vs Luminal B)
Conclusions: 1. Luminal A type BCa is associated with better prognosis (NPI >5.4) when compared with Luminal B type BCa (38 vs 19%). 2. In the intermediate prognostic group NPI 3.4 to 5.4, the number of Ki-67 and p53 positive tumors is significantly higher in luminal B type (p<0.05) than type A BCa (data not shown). 3. The Her2 status of luminal A type BCa with high Ki-67 and p53 should be reconsidered.

125   Invasive Micropapillary Breast Carcinoma Is a Lymphotropic Entity That Belongs to the Luminal Group. A Phenotypic, Chromogenic *In Situ* Hybridization and Retrospective Study of 164 Cases
F Bibeau, H Frugier, F Mishellany, G Mac Grogan, M Lacroix-Triki, F Penault-Llorca, M Antoine, V Becette, P Berteau, F Boissiere-Michot. CRLC Val d'Aurelle, Montpellier, France; Centre Jean Perrin, Clermont-Ferrand, France; Institut Bergonié, Bordeaux, France; Institut Claudius Regaud, Toulouse, France; Hopital Tenon, Paris, France; Centre Rene Huguenin, Saint-Cloud, France; Hopital Saint-Louis, Paris, France.
Background: Invasive micropapillary breast carcinoma (IMBC) is an aggressive variant of breast cancer, characterized by a specific "inside–out" pattern and lymphotropic propensity. The phenotypic and molecular categories in which IMBC could be included are still subject of investigation.

Design: The aim of our study was to assess the pathologic features of 164 IMBC and to specify their phenotype by immunohistochemistry (IHC) on tissue-microarray. IHC was performed with the following markers: (i) luminal: Estrogen and Progesterone receptors (ER, PR), cytokeratins (CK)7, CK8-18; (ii) HER2 (*HER2* chromogenic *in situ* hybridization (CISH) on any HER2 immunoreactive case); (iii) basal/ myoepithelial: CK5/6, CK14, EGFR, vimentin, CD117; p63, SMA, S100; (iv) others: Epithelial membrane antigen (EMA), Ki67.

Results: Multifocality was observed in 21% of IMBC. IMBC were pure, predominant (>50%) or focal (<50%) in 50, 32 and 18% of the cases, respectively. SBR grades II, III and I were observed in 66, 29 and 9% of IMBC, respectively. Despite a majority of pT1 stage (63%), 66% of IMBC displayed lymph node invasion and 70% had lymphatic invasion. All tumor cell clusters showed a typical EMA peripheral staining. A luminal phenotype with no basal marker expression was observed in 83% of IMBC, of which 15% presented a Ki67 immunostaining ≥20%. *HER2* amplification was observed in 12% of IMBC, surprisingly associated in 5 cases with an incomplete membrane immunostaining. Two percent of IMBC had a basal phenotype (ER-, PR- , *HER2* non amplified, basal markers +), while 3% of IMBC were triple negative and did not express basal markers. No myoepithelial marker was expressed.

Conclusions: IMBC mainly belongs to the luminal group and is an agressive entity, with a high proportion of lymphatic and lymph node invasion. In order not to miss patients who would benefit from trastuzumab therapy, assessment of *HER2* gene status should be performed in all IMBC, due to the peculiar HER2 immunoreactivity associated with amplification in this special type of breast cancer.

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126   Quantitative Assessment of Her-2 mRNA by PCR in Breast Carcinomas Failing To Hybridize with HER-2 Fish Probes
KJ Bloom, P Choppa, TA Bloom, A Kyshtoobayeva, M Hibbard. Clarient, Aliso Viejo, CA.

Background: The determination of HER-2 status is essential to aid the oncologist in the selection of an appropriate chemotherapeutic regimen. Although the failure rate of FISH is relatively low, 2-4% in most reported series, failure to hybridize still occurs several times per day in a high volume laboratory. In addition, specimens exposed to acids or bases or inappropriate fixatives also render them unable to hybridize. We hypothesized that the quantitative assessment of HER-2 mRNA in this setting may be possible. After establishing that HER-2 mRNA levels correlate tightly with HER-2 protein expression and gene copy number, we assessed 198 consecutive breast carcinomas that failed to hybridize with the PathVysion FISH assay by quantitative PCR.

Design: One hundred ninety eight consecutive breast carcinomas which failed to hybridize with the PathVysion HER-2 FISH assay (Abbott) were submitted for PCR testing. Tissue sections were cut at 7 microns and mounted on uncharged slides. The samples were stained with H&E using a Leica Autostainer (Meyer Instruments, Houston, TX). Cells of interest were identified and removed from the sections using a PALM laser capture microdissection system (Zeiss, Jena, Germany). RNA was isolated from the captured cells using RNeasy mini kit (Qiagen, Valencia, CA). Gene expression analysis was performed using one step real-time RT-PCR chemistry on an Applied Biosystems 7900HT (ABI, Foster City, CA). Standard curves were generated from universal RNA to determine the relative expression of HER-2 and GUSB. The HER-2 quantitative values are expressed as a ratio to GUSB.

Results: One hundred fifty four of the 198 tumors analyzed (78%) yielded a quantitative PCR result. One hundred twenty three (80%) revealed low expression of HER-2 mRNA (less than 30% relative to the reference gene), 10 (6.5%) revealed borderline expression of HER-2 mRNA (30-40% relative to the reference gene) and 21 (13.5%) revealed high expression of HER-2 mRNA ranging from (42% to 491% relative to the reference gene).

Conclusions: Assessment of HER-2 mRNA has been shown to tightly correlate with HER-2 protein expression and HER-2 gene copy number. The expression of HER-2 mRNA could be reliably determined in 78% of samples which failed to hybridize with the PathVysion HER-2 FISH assay with approximately 80% of samples lacking significant over-expression . HER-2 mRNA determination may be a viable alternative when HER-2 FISH probes fail to hybridize.

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127   HER2 2+ Breast Cancer in Mexico, Central America and the Caribbean: Re-Testing Results by a Central Lab
OL Bohn, L Navarro, C Ayaquica, J Saldivar, S Sanchez-Sosa. UPAEP University Hospital, Puebla, Mexico.

Background: Pathologists have a prophetic role in determining Trastuzumab use. Concordance studies between central and local laboratories in North America and Europe have shown high degree of discordance, mostly in 2+ equivocal cases. No data of HER2 quality assurance are available from Latin America and the Caribbean. We determine concordance HER2 IHC 2+ in invasive ductal breast carcinomas cases between 29 local laboratories (15 in Mexico, 10 in Central America and 4 in the Caribbean) and a central reference laboratory in Mexico. In local laboratories, HER2 2+ tumors range between 6 to 11% of the total of cases submitted for HER2 IHC testing.

Design: A total of 1,086 blocks were reviewed by the central laboratory, interpreted by the local laboratories as 2+. Thirty-seven blocks were excluded (no tissue, no tumor present in the block). All tumor specimens were retested by HER2 IHC (Hercep Test) using the DAKO scoring system and fluorescence in situ hybridization (FISH). International external quality assurance to the central laboratory was run by the UK National External Quality assessment scheme (UK-NEQAS).

Results: Concordance between local and central laboratories HER2 IHC 2+ was 29.08% (n=305). Among 1,049 tumors, 539 (51.38%) were re-classified by the central lab as 0/1+ and 205 (19.54%) as 3+.

Table 1. Re-testing IHC by central laboratory

IHC	FISH NON-AMPLIFIED	FISH EQUIVOCAL	FISH AMPLIFIED	TOTAL
0/1+	518	6	15	539 (51.38%)
2+	155	26	124	305 (29.08%)
3+	2	2	201	205 (19.54%)
TOTAL	675	34	340	1049

HER2 gene amplification by FISH, performed by central lab (HER2/CEP17>2.2), was present in 124/1,049 (11.82%) of the cases that had been reported as 2+ in the local laboratories, and equivocal amplification (HER2/CEP17 1.8-2.2) was present in 26/1,049 (2.48%). Concordance between central IHC and central FISH ranged between 96.10% (0/1+) and 98.05% (3+).

Conclusions: Current HER2 concordance for IHC 2+ equivocal cases between local and central laboratories is low, and in agreement with ASCO/CAP guidelines, HER2 testing should be performed by high-volume reference laboratory.

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128   Clinical Implications of Intra-Tumoral Variation in HER2 Expression in Breast Cancer
M Bonham, A Lal, S Devries, E Hwang, J Rabban, F Waldman, Y Chen. UCSF, San Francisco, CA.

Background: HER2 is overexpressed in ~ 20% of invasive breast carcinomas. Previous studies have shown that HER2 status is similar between in situ and invasive components of the same tumor and that there is high concordance for HER2 expression between primary breast cancer and its corresponding metastases. Little is known about the intratumoral variation of HER2 status in breast cancer. This study examines the intratumoral heterogeneity of HER2 and explores the possible clonal evolution of this phenotype.

Design: We reviewed a database of 893 primary breast cancer cases from 2005-2008 and found 11 cases of breast cancer with at least two distinct patterns of differing intensity in HER2 expression by immunohistochemisty. The intratumoral variation in HER2 among the 11 cases was examined by FISH (Vysis) and CISH (Zymed) analysis. The presence of clonal relationship between the variable regions of HER2 expression will be assessed by array comparative genomic hybridization (aCGH).

Results: Ten of the 11 cases showed markedly different levels of HER2 expression among invasive and in situ components within the same tumor. Four invasive ductal carcinomas had two distinct levels of HER2 expression. Six in situ carcinomas showed different levels of HER2 expression. FISH confirmed the amplification status and difference in expression among the eleven samples. CISH morphologically correlated the variation in gene amplification to the histopathology. The clonal relationship and evolution of these lesions with different HER2 will evaluated by aCGH.

HER Heterogeneity in Components of Breast Cancer

Case	1	2	3	4	5	6
Invasive	+,-	+,-	+,-	+,-	-	-
In situ	+	+,-	+	+	+	+
Case	7	8	9	10	11	
Invasive	+	+	+	-		
In situ	+,-	+,-	+,-	+,-	+,-	

+,- indicates HER2 status. All cases are invasive ductal carcinoma with DCIS except cases 9 &10 which are invasive lobular carcinoma with LCIS. Metastasis in case #1 was HER2 neg.
+,- indicates HER2 status. All cases are invasive ductal carcinoma with DCIS except cases 9 &10 which are invasive lobular carcinoma with LCIS. Metastasis in case #1 was HER2 neg.

Conclusions: Intratumoral variation in HER2 expression is a rare event. However, the possibility of HER2 variation is clinically significant in that treatment decisions are increasingly made based on HER2 studies after analysis of a limited sampling of tumor such as core needle biopsy, fine needle aspiration, or by molecular analysis of tumors that may contain both invasive and in situ components with distinct subpopulations. In some cases, the different areas of HER2 expression in invasive component appear to be derived from the in situ component, however, the cause for the HER2 expression change and potential effect on treatment outcome is unknown.

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129   Patterns and Utility of Myoepithelial Cell Staining in Ductal Carcinoma In-Situ with Questionable Invasive Mammary Carcinoma
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Background: Differentiating ductal carcinoma in-situ (DCIS) from invasive carcinoma (IMC) is a recurring issue in breast pathology, and one that potentially carries significant therapeutic and prognostic implications. The tools that have provided greatest assistance in this distinction are immunohistochemical markers of myoepithelial cells (MEC) which are normally present around DCIS and lost in IMC. The expression of MEC markers is not always uniform, however. The purpose of this study is to evaluate myoepithelial cell staining patterns in DCIS and IMC among cases with questionable invasion and hence assess the utility and potential pitfalls of this diagnostic adjunct.

Design: Fifty-nine consecutive cases of DCIS that required resorting to MEC stains for purposes of final diagnosis were retrieved from the files of the Breast Consultation Service at Vanderbilt University Medical Center. All cases were stained for smooth muscle actin (SMA) and p63. Twenty-five cases with available unstained slides were also stained for Smooth Muscle Myosin Heavy Chain (SMMHC) and CD10. Staining was evaluated relative to internal and external controls.

Results: 8 out of 57 cases (14%) did not show any staining in either in-situ or invasive areas. The DCIS in these cases was low-grade (n=3), mixed low and intermediate grade (n=2), intermediate grade (n=2), and high grade (n=1). The DCIS was involving clustered or multiple papillomas in five of eight cases. Twenty-one other cases (37%), five of which containing involved papillomas, had at least discontinuous staining with focal ducts completely negative for MECs. As far as individual stains were concerned, p63 was less often positive compared to SMA, SMMHC and CD10. The latter two mirrored SMA's staining pattern and intensity with little non-specific myofibroblastic staining. Overall, the MEC immunohistochemical stains unequivocally confirmed the

diagnosis in 21 of 57 cases. Three cases remained inconclusive and the remaining cases were diagnosed based on the overall Hematoxylin and Eosin impression with marginal help from immunostains.
Conclusions: Myoepithelial stains can be decreased or completely absent in a significant proportion of DCIS. Hence extreme care should be exercised when dealing with suspicious foci in order not to overdiagnose nor overestimate the extent of invasion based on a negative immunostain.

130 Basal-Like Ductal Carcinoma In Situ Is a Precursor Lesion for Invasive Metaplastic Breast Carcinoma
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Background: Metaplastic breast carcinoma (MBC) is an uncommon variant of breast cancer that typically demonstrates a triple-negative phenotype (ER-, PR-, HER2-). Multiple studies have documented the presence of an in situ counterpart of basal-like breast carcinoma. Detailed studies characterizing the precursor lesions of invasive MBC are lacking. The aim of this study was to evaluate the histopathology and immunophenotype of ductal carcinoma in situ (DCIS) associated with MBC and to determine whether the DCIS demonstrates a basal-like phenotype.
Design: Review of UNC surgical pathology files from 1997 to 2008 revealed 20 cases of MBC with accessible slides and blocks. Complete histopathologic review of these cases was performed to document histopathologic features of the invasive and, if present, in situ components including nuclear grade, presence of necrosis, and presence/type of metaplastic features. Immunohistochemistry (IHC) for ER, HER2, EGFR and cytokeratin (CK) 5/6 was performed on all cases with an in situ component. Basal-like tumors were defined as those showing an ER-, HER2-, and CK5/6+ and/or EGFR+ immunophenotype.
Results: A total of 9 of 20 cases demonstrated an in situ component associated with the invasive MBC. The histologic differentiation of the invasive component in these 9 cases included 5 squamous, 2 squamous and sarcomatoid, 1 chondroid, and 1 chondroid and sarcomatoid carcinomas. The associated DCIS was high nuclear grade 3 with necrosis in all 9 cases. The DCIS foci did not show associated metaplastic changes in 8 of 9 cases. One of the 9 cases showed focal squamoid differentiation within the DCIS. Extent of DCIS ranged from rare foci (<1%) to extensive (>75%) of total tumor volume. All nine cases exhibited a basal-like phenotype in the invasive component. Immunohistochemical results for all four markers were able to be interpreted for the DCIS component in 7 of 9 cases. All 7 cases demonstrated a basal-like DCIS phenotype, each showing an ER-, HER2-, CK5/6+ and EGFR+ phenotype.
Conclusions: Basal-like DCIS is associated with MBC and is a likely precursor lesion for this rare subtype of invasive carcinoma. DCIS associated with MBC is typically high-grade and often lacks the characteristic metaplastic changes seen in the associated invasive component. Identification of metaplastic changes within expansive DCIS in biopsy specimens should raise the suspicion for the presence of invasive carcinoma.

131 Clinicopathologic Characteristics in Correlation with Protein Expression Profile in Triple Negative Breast Cancer in Our Population
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Background: Triple negative tumors (TNT) of the breast are ER, PR and Her negative and represent a group of tumors with no benefits from specific therapy. They are more frequent in younger women and portend a worse outcome. We studied clinico-pathological features and markers associated with the triple negative phenotype.
Design: Tissue microarray (TMAs) from 125 breast carcinomas diagnosed during a 6 years period. 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, Topoisomerase 2, Survivin, c-Kit, p53, p63, androgen receptor (AR) and Zeb. Clinical pathologic data, including race, age, tumor size, tumor grade, TNM stage and follow-up was obtained. Statistical analysis was performed using chi square analysis and linear regression with a p value of 0.05 or less significant.
Results: Of the 135 patients, 107 were African-American (AA), 18 Caucasians (CS), 6 other and 4 unknown. We further analyzed the data for the AA and CS groups. The age, pathology and the IHC statistically significant data are presented in table 1.

	African-Americans 107 (85.6%)	Caucasians 18 (14.4%)	
Age range	23-83	18-80	
Age average	50	45	
Age less or 50-years			
Age older than 50-year			
T1	17	6	
T2	69	4	
T3	21	2	0.016*
N0	25	5	
N1	20	6	
N2	11	6	
N3	1	0	0.488
CD44	56/93	1/17	0.007*

* Denotes statistical significance. All other IHC stains did not show statistical significance by chi square
In addition CD44 and EGFR were more common in the AA population. CK7, p-cadherin and race were predictive of T stage. CK14 and survivin were predictive for N stage.
Conclusions: 1. In our population we found no statistically significant inter-racial age difference. 2. In regards to TNM elements, there was a statistically significant difference in the tumor size, while such a difference was not noted in lymph node status. 3. Within the large panel of IHC we performed, statistical differences were found in CD44 and EGFR expression, both more frequently found in the AA population. As CD44 is a putative marker for breast stem cells, further investigation is warranted in this direction.

132 The Effects of Additional Tumor Cavity Sampling at the Time of Breast Conserving Surgery on Final Margin Status, Volume of Resection, and Pathologist Work-Load
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Background: The effect of removing additional tumor cavity margins at the time of initial breast conserving surgery (BCS) have not been demonstrated. A few studies suggest that there is a reduction in the re-operation rate for microscopic presence of tumor at the margin. This study compares margin status, volume of breast tissue excised, and pathologist work-load in two groups of patients who underwent BCS with or without resection of 4 or 5 additional margins (BCS+M).
Design: We retrospectively analyzed 320 patients who underwent BCS or BCS+M for ductal carcinoma in situ (DCIS) or infiltrating ductal carcinoma (IDC) from 2004 to 2007. Based on the distance from the tumor to margin of resection we classified the margins as negative ($\geq$1 mm), close (< 1 mm) or positive (tumor cells at inked margin). Volume of breast tissue excised was calculated in cm^3 from the tri-dimensional size recording in the pathology report. Also, the number of submitted blocks per case was extracted from the pathology report. Data were statistically analyzed using Fisher's Exact and Wilcoxon rank sum test.
Results: Of 320 cases analyzed, 199 (62.2%) underwent BCS and 121 (37.8%) had BCS+M. Overall, patients with BCS+M had a higher negative margins rate (89.3%vs 75.4% p<0.05). Also, there was no overall difference in total volume of breast tissue excised. However, when DCIS and IDC were analyzed separately, only patients with IDC showed a higher negative margin rate (p< 0.001) and a lower volume of breast tissue excised (p =0.03). For DCIS, there was no difference in margin status and volume of breast tissue removed with either type of surgery. Also, there was no significant difference in number of submitted blocks for cases with positive/close margins.
Conclusions: This study suggests that resection of 4-5 additional margins during BCS for early stage invasive breast cancer significantly reduces rate of positive margins while there is no advantage in such surgical approach for DCIS. Interestingly, for BCS + M, this study suggests that the volume of tissue resected for cases of IDC was actually statistically significantly decreased; however, there was no overall significant difference in the volume of tissue resected both for DCIS and IDC. Also, comparison of the number of blocks submitted suggests that surgery with additional margin resections does not significantly increase the pathologist work-load or time spent per case.

133 Differential Localization of EZRIN Expression in Normal and Neoplastic Mammary Tissue
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Background: The cytoskeletal plasma membrane cross-linker protein EZRIN is required for polarity of normal breast epithelium. Interestingly, loss of EZRIN function inhibits metastasis with little effect on primary growth of breast carcinoma cells in a xenograft model (Elliott *et al.*, BCR 7:R365-73, 2005). Our goal was to examine the role of EZRIN and other associated signaling markers in a cohort of primary invasive breast cancers from premenopausal women and to explore if they are correlated with established prognostic factors.
Design: Using a triplicate core TMA of formalin-fixed tissue, we investigated sixty-two primary invasive breast cancers from women under 49 years of age. Immunohistochemistry was performed on the TMA for PAN-EZRIN and its modulators (E-CADHERIN, MET, SRC, STAT5), cell cycle/apoptosis regulators (BCL2, CYCLIN D1, P53), HER2/neu, ER and PR. The percentage of positive cells and staining intensity were scored by two independent evaluators with resolution of discordant cases by a senior Pathologist. The statistical analysis included *Pearson* and *Spearman* correlation coefficients.
Results: Forty-four infiltrating ductal and lobular carcinomas were analyzed. About half of invasive carcinomas (54.5%) displayed diffuse cytoplasmic PAN-EZRIN staining whereas the remaining half showed absence of staining. PAN-EZRIN expression was limited to the apical membrane in normal mammoplasty tissue (8%). Neither cytoplasmic nor membranous PAN-EZRIN was significantly associated with tumor size, nodal status, ER, PR and HER/neu expression (*p*>0.10).
Conclusions: We found unique localization of EZRIN staining in a sub-population of breast cancers. The fact that EZRIN plays a key role in cell adhesion, migration and division coupled with our finding that EZRIN staining did not correlate with established prognostic factors makes it tempting to speculate that EZRIN staining pattern may be an independent prognostic factor (Supported by Queen's Pathology Clinical Trust and CBCRA).

134 The Incidence of Invasive Carcinoma and Ductal Carcinoma In Situ Following a Core Biopsy Diagnosis of Lobular Neoplasia
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Background: Lobular neoplasia (LN), encompassing atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS), is generally regarded as a risk factor for the subsequent development of invasive carcinoma (IC) in either breast. The clinical management of patients with a core biopsy (CB) diagnosis of LN remains the subject of debate. This dilemma is becoming increasingly common as increasing numbers of image directed CBs are performed for a variety of mammographic and ultrasound findings. Previous studies on this topic have yielded conflicting results and have been confounded by the inclusion of other lesions [e.g., atypical ductal hyperplasia (ADH)] in some of the cases. The aim of this study was to review our institutions incidence of carcinoma on follow-up excisional biopsy (EB) after a CB diagnosis of LN.
Design: 67 breast CBs with a diagnosis of LN, ALH or LCIS, without IC or DCIS, were retrieved from the surgical pathology files of Hackensack University Medical Center

from Jan. 1998 to the present. 19 cases were excluded because of the presence of ADH. Of the remaining 48 cases, 13 had a subsequent EB performed within 3 months of the CB. All available slides were reviewed from the CB and the subsequent EB.

Results: Of the 13 CBs examined, 8 were performed for calcifications, 2 for a mass, 2 for a nodule and 1 for an abnormal mammogram. The follow-up EB revealed 2 cases of DCIS, 1 invasive ductal carcinoma (IDC) and 1 invasive lobular carcinoma (ILC). The mammographic findings of the 4 patients with carcinoma were as follows: calcifications alone (DCIS), mass with calcifications (IDC, ILC), and architectural distortion with calcifications (DCIS). The overall incidence of carcinoma following a CB diagnosis of LN is 4/13 (31%).

Conclusions: Our data corroborates the high incidence of carcinoma seen in previous studies on this topic. Since the carcinomas occurred in women with calcifications and in those with a mass, these results strongly support a management strategy advocating EB in all patients with a CB diagnosis of LN.

135 Grap2 and Cyclin-D Interacting Protein (GCIP) Nuclear Expression Is Significantly More Frequent in Triple-Negative Breast Carcinomas (TNBCs) than Other Subtypes of Breast Carcinomas
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Background: GCIP is a putative tumor suppressor in human carcinomas. GCIP exerts its function in the nucleus. In vivo studies showed GCIP bound to cyclin D1, reduced the phosphorylation of retinoblastoma protein and leaded to inhibition of the transcriptional activity mediated by E2F1 protein. Using immunophenotypes, molecular subtypes of breast carcinomas can be divided into four groups: "luminal A" (ER+ and/or PR+, HER2-), "luminal B" (ER+ and/or PR+, HER2+), "HER2" (ER- and PR-, HER2+) and "TNBC" (ER-, PR-, HER2-). Of these subtypes, TNBC is a distinct group with poor prognosis and currently lacks targeted therapies. In this study, we compared GCIP nuclear expression using immunohistochemistry in the four subtypes of breast carcinomas.

Design: Fifty-three cases of primary invasive breast carcinomas were retrieved from archival files at our institution. Cases were further subdivided into "luminal A", "luminal B", "HER2" and "TNBC" as defined above. The status of HER2 was determined by immunohistochemistry and followed by fluorescent in situ hybridization if the immunohistochemical results were equivocal. Immunohistochemical staining using GCIP monoclonal antibody was performed on whole tissue sections of all cases.

Results: Of 53 primary breast carcinomas, 29 (54.7%) were "luminal A", 10 (18.9%) were "luminal B", 4 (7.5%) were "HER2" and 10 (18.9%) were "TNBCs". GCIP nuclear staining was observed in 5 of 29 (17.2%) "luminal A" carcinomas, 1 of 10 (10%) "luminal B" carcinomas, none of 4 (0%) "HER2" carcinomas and 7 of 10 (70%) "TNBCs". TNBCs had significantly more frequent GCIP nuclear staining than other subtypes of breast carcinomas (p=0.002).

Conclusions: A significant percentage of TNBCs have GCIP nuclear expression compared to other subtypes of breast carcinomas. Contrast to other subtypes, the tumorigenesis of TNBCs may not involve inactivation of GCIP.

136 The Role of Sentinel Node (SN) Mapping in Patients with High Grade Ductal Carcinoma In-Situ (HG-DCIS) Diagnosed by Needle Core Biopsies (NCB)
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Background: The current practice recommends SN mapping in all HG-DCIS patients. While this may be indicated in simple mastectomies, concomitant SN mapping in breast conservation remains controversial.

Design: Mammographic, sonographic and magnetic resonance imaging (MRI) data from 95 consecutive patients with initial diagnosis of HG-DCIS only, HG-DCIS suspicious (susp) for microinvasion (Tmic) and HG-DCIS with Tmic disease on NCB were reviewed. Mammographic data included: microcalcifications (Ca++) only versus Ca++ with associated masses/densities, and multifocal versus unifocal disease. Sonographic data was recorded as positive or negative for associated masses. MRI results, when available, were used to evaluate extent of disease and multifocality. All patients underwent SN mapping at the time of their breast surgical procedures (mastectomies or lumpectomies). NCB results were then correlated with the results of the definitive surgical procedures.

Results: The overall incidence for metastatic disease in patients with predominantly HG-DCIS in NCB was 9.4% (9/95). The incidence of metastatic disease in the individual categories was as follows: 1) HG-DCIS only = 2.5 % (1/40); 2) HG-DCIS susp for Tmic: 6.6 % (2/30) and 3) HG-DCIS + Tmic: 24% (6/25). All patients with metastatic disease were N1a, and in retrospect, all had either mammographic, sonographic or MRI breast findings suspicious for invasive carcinoma. Excluding 2 patients with multifocal invasive lobular carcinoma, metastases were associated with high grade primaries > 0.5 cm. None of the patients presenting with microcalcifications without (w/o) associated masses or densities had metastatic disease. The extent of the microcalcifications and evidence of multifocality correlated with increased incidence of Tmic or invasive disease, but not necessarily with metastatic disease. Breast conservation patients had a lesser incidence of metastatic disease (n = 1) than patients undergoing mastectomies for reasons other than cosmetic results (n = 8).

Conclusions: For breast conservation patients with radiographic data showing pleomorphic Ca++ w/o associated masses and NCB diagnoses of HG-DCIS only or HG-DCIS susp for Tmic, SN mapping should be offered only after confirmation of invasive disease.

137 Differential Pattern of Methylation within Hormone Receptor Negative Breast Cancers
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Background: Like all cancers, breast cancer is considered to result in part from the accumulation of multiple genetic alterations leading to oncogene overexpression and tumor suppressor loss. The role of epigenetic tumor suppressor gene silencing by promoter methylation in many genes is increasingly recognized as an early event in carcinogenesis. Our aim was to explore pattern of promotor methylation in a set of tumor suppressor genes in hormone receptor negative breast cancers.

Design: From a cohort of 196 ER-PR- breast cancers from 2001 to 2005 (156 triple negative tumors (TNT) and 40 Her2+), 30 cases (22 TNT, 8 Her2+) were randomly selected for this pilot methylation project. Formalin fixed paraffin embedded tumor DNA was interrogated for 24 tumor suppressor genes using the high throughput Methylation-Specific Multiplex Ligation Dependent Probe Amplification (MS-MLPA). All H & E slides were reviewed and the tumors were classified into medullary, atypical medullary (AMC) and non-medullary (NMC)subtypes and results compared with the methylation data.

Results: Promotor methylation was noted in 21/24 genes: *TIMP3, APC, CDKN2A, MLH1, RARB, CDKN2B, HIC1, BRCA1, CASP8, PTEN, BRCA2, CD44, RASSF1, DAPK1, VHL, ESR1, TP73, FHIT, IGSF4, CDH13, GSTP1*. The most frequently methylated genes were *MLH1*-16/30 (53.3%), *RASSF1*-15/30 (50%), *CDH13*-12/18 (40%), *CDKN2B,GSTP1*-9/30 (30%), *CDKN2A* (26.7%), *BRCA1, BRCA2,RARB*-6/30 (20%). *CDKN1B, ATM*, and *CHFR* genes were unmethylated in this group. *RASSF1* was significantly methylated among the Her2+ group-7/8 (88%) compared to the TNT-8/22 (36%) (p=0.035). In contrast, *BRCA1,BRCA2*-6/22 (37.5%) were frequently methylated in the TNT subgroup (trend, p= 0.155). Only one case of methylated *BRCA1* was co-methylated with *BRCA2*, others being mutually exclusive. All the 8 Her2+ cases had unmethylated *BRCA1/2*. There were 8/30 AMC (all TNT) and 22 NMC (8/22 Her2+). No correlation was observed between histology and methylation patterns.

Conclusions: Breast cancer is a heterogeneous disease with different outcomes, TNT's having an adverse prognosis. The emerging differential methylation pattern within hormone receptor negative breast cancers would further help stratify them into distinct subgroups. Promotor methylation being potentially reversible, methylated genes may serve as future molecular targets for demethylating therapies. Support: NIH R01 DE15990.

138 'Flat Epithelial Atypia': Impact of the Entity with Reference to Number of Levels Obtained on the Paraffin Embedded Blocks of the Breast Core Needle Biopsies
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Background: Flat epithelial atypia (FEA) is a newly emerging entity of uncertain clinical significance. Defined by the World Health Organization (WHO) in 2003, FEA differs from columnar cell hyperplasia by the presence of nuclear atypia and from atypical ductal hyperplasia (ADH) by the absence of complex architecture. As the term FEA is relatively new, the clinical relevance and the outcome data are sparse. The aim of this study was to evaluate the pathologic significance of FEA with reference to number of levels obtained on the paraffin blocks of the core needle biopsy samples (CNB).

Design: All core-needle biopsies (CNB) diagnosed as ADH, which includes pure FEA at our institution, from January 2006-April 2008 were retrieved from our pathology files. Hematoxylin and eosin (H&E) slides of five levels on each case were reviewed. Statistical analysis was performed with significance defined as p-value of <0.05.

Results: Total number of CNB performed from 2006-2008 was 8054. Ninety nine percent **99%** (8051/8054) were stereotactic guided and 1% (3/8054) was ultrasound/MRI guided biopsies. All the CNB were performed for microcalcifications. The mean age of the patients is 54 years (range 29-83). Incidence of ADH (including FEA) was **4%** (338/8054). Slides from 203 cases were available for review. 32 cases were discarded due to either presence of DCIS or IC in the CNB. Upon review, **9%** (18/203**)** cases were classified as pure FEA and **91%** (185/203**)** cases as FEA+ADH. In **6%** (11/185) of cases in FEA+ADH group, we observed FEA evolved into ADH at the same site at an average of 3-4 levels. The upstaging to a more clinically significant lesion in pure FEA group is **14%** in comparison to **12%** in FEA+ADH group (**p=0.9471**). Lobular neoplasia seen in association with pure FEA group in **33%** (6/18) and **11%** (21/185) in FEA+ADH group.

Conclusions: 1. The incidence of FEA in our CNB targeted for calcifications is **9% 2.** Since FEA and ADH commonly occur together on the same slide, it is prudent to examine deeper tissue levels when pure FEA is encountered on CNB. **3.** The upstaging in the follow-up resections in FEA in comparison to ADH+FEA shows no statistical difference.

139 Expression of Estrogen Receptor-Beta and Its Isoforms in Breast Cancers
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Background: ER-β has several isoforms and some of them have shown to be associated with unfavorable outcomes. Previously, our study showed ER-β wild type (wt) expression in ER-α negative tumors. This study aims to test expression of ER-β wt and its isoforms in triple negative (TN) and tamoxifen resistant (TR) breast cancers.

Design: A tissue microarray sldies from 226 breast cancers were tested for the expression of ER-α (Dako. CA), PR (Dako, CA), HerceptTest kit (Dako, CA), ER-β wt (Biogenex, CA), ER-β1(Dako, CA), ER-β2/bcx(AbD serotec, UK), ER-β,CT(Lake Placid, NY), ER-beta NT(Millipore, MA) and ER-β1(AbD serotec,UK) using standard immunohistochemistry procedures. They were also analyzed for histopathology and associations between ER-β andp53 and Ki-67. More than10% nuclear reaction was considered positive.

Results: The 226 breast cancers consisted of 20 stage 0 cases (9%), 20 stage I cases (9%), 63 stage II cases (28%), 23 stage III cases (10%), and 100 stage IV cases (44%) which included 63 patients who were treated with tamoxifen but developed stage IV disease. ER-α negative and TN cancers comprised of 55.4% (124/226) and 20.8% (47/226), respectively. TN cancers consisted of grade 3 in 74.5%(35/47), ductal type in 59.6% (28/47), basaloid in 5.5% (12/47), apocrine type in 8.5% (4/47) and metaplastic changes in 2.1% (1/47).TN cancers showed expression of ER-β wild (wt), ER-β2/bcx, and ER-β1 in 57.8% (26/45), 63.6% (28/44), and 64.4% (29/45), respectively and co-expression of all three ER-β types in 51.2% (22/43) and no ER-β expression in 2.3% (1/43).TR cases showed expression of ER-β wt, ER-β2/bcx and ER-β1 in 50.8% (32/63), 49.1% (30/61), and 54.7% (33/61), respectively. P53 expression was present in 27.7% (13/47) of TN and 33.4 %(21/62) of TR cases compared to 34.5% (78/226) of the total. Ki-67 expression was in 6.4% (3/47) of TN and 12.3% (8/62) of TR versus 18.1% (41/226) of total cases. Ki-67 co-expressed with ER-beta wt in 92.6% (38/41), with ER-β1 in 97.6% (40/41), and with ER-β2 in 95.1% (39/41) of TN and with ER-β wt in 100% (8/8), ER-β1 in 100% (8/8), and ER-β2 in 62.5%(5/8) of TR.

Conclusions: ER-βwt, ER-β1 and ER-β2/bcx are frequently expressed in TN and TR breast cancers. A significant correlation between expression of the three ER-β types and Ki-67 expression suggests that both TN and TR have ER-β positive proliferating cancer cell, not responding to the treatment. Both ER-β1 and ER-β2 expression were similar.Presence of ER-β in this sub-population of breast cancers may have significant clinical and therapeutic implication, and ER-β may be involved in estrogen signaling in these cancers.
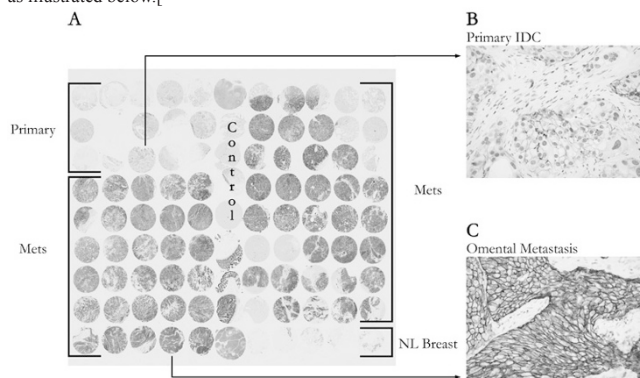
140 Epithelial Cell Adhesion Molecule (EpCAM) Is Overexpressed in Breast Cancer Metastases Compared to Matched Primary Cancers

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Background: The transmembrane protein EpCAM has diverse roles in cell adhesion, proliferation, and migration and is overexpressed in breast carcinoma relative to normal breast epithelium. While preclinical data suggest a role for EpCAM in metastases, a study of breast cancer micrometastases to bone marrow (Clin Cancer Res 2003; 9:2598-2604) suggested that EpCAM expression is decreased after first-line chemotherapy. No prior studies have compared EpCAM expression in metastatic breast carcinoma (MBC) versus matched primary breast carcinoma (PBC).

Design: Fifteen rapid autopsies (post mortem intervals <4 hours) were performed on patients who died of MBC. Single patient tissue microarrays (TMAs) were constructed from paraffin tissue blocks of patients' archived PBC and from multiple MBCs sampled at autopsy. Fifteen TMA slides containing 145 spots of PBCs and 778 spots derived from 180 different MBCs were labeled by immunohistochemistry for EpCAM. EpCAM expression was scored as labeling intensity (1-3) multiplied by percent membrane labeling (0-100%). Each patient's average PBC and MBC labeling score was subclassified as low (score 20-100), moderate (score 101-200), or high (score 201-300). Quantitative reverse transcription-polymerase chain reaction (QRT-PCR) was performed on microdissected unmatched PBCs and MBCs to quantify EpCAM mRNA expression.

Results: All PBCs and MBCs demonstrated membranous EpCAM labeling. PBCs exhibited low to moderate average EpCAM labeling. EpCAM labeling was statistically significantly increased in MBCs compared to matched PBCs in 12 of 15 patients (80%; p values ranging from <0.05 to <0.0001, t test), and strikingly so in 4 patients as illustrated below.[

In the remaining 3 patients, EpCAM labeling was nonsignificantly increased in 1 and unchanged in 2. In all cases, EpCAM expression was uniform in all MBCs. Surprisingly, QRT-PCR did not show upregulation of EpCAM mRNA transcripts.

Conclusions: EpCAM is consistently upregulated in MBCs as compared to matched PBCs. The absence of increased RNA expression suggests a post-translational mechanism for protein overexpression. EpCAM is a promising therapeutic target for MBC.

141 Relationship between Molecular Phenotype of Invasive Breast Cancer and Expression of Androgen Receptor

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Background: Distinct subsets of invasive breast cancer have been identified by their patterns of gene expression and include luminal (A and B), HER2 and basal-like types.

While prior studies have demonstrated that androgen receptor (AR) is expressed in many breast cancers, the frequency of AR expression in relation to the various breast cancer subtypes defined by molecular analysis has not been previously studied in detail.

Design: We constructed tissue microarrays (TMAs) from paraffin blocks of 3,093 breast cancers that developed in women enrolled in the Nurses' Health Study between 1976-1996. TMA sections were immunostained for ER, PR, HER2, CK5/6 and EGFR. Results of these stains were used to categorize each cancer as luminal A (ER+ and/or PR+ and HER2-); luminal B (ER+ and/or PR+ and HER2+); HER2 (ER- and PR- and HER2+); basal-like (ER-, PR-, HER2- and EGFR or CK5/6+); or unclassified (negative for all markers). TMA sections were also immunostained with a monoclonal antibody to AR. The relationships between AR expression and molecular subtypes were analyzed.

Results: There were 1,990 invasive cancers with complete immunophenotypic data: 73% were luminal A, 5% luminal B, 6% HER2, 11% basal-like and 5% unclassified. Overall, 77% of cases were AR-positive but the frequency of AR expression differed significantly across the molecular phenotypes (p<0.0001). AR expression was commonly observed in luminal A and luminal B cancers (88% and 72% of cases, respectively), but was less frequently seen in HER2 cancers (60% of cases). Moreover, despite being defined by the absence of ER and PR expression and being considered as hormonally unresponsive, 32% of basal-like cancers showed expression of AR.

Conclusions: AR expression is most commonly seen in the luminal A- and B-types of invasive breast cancer. However, expression of AR is also seen in approximately one-third of basal-like cancers, despite the absence of ER and PR expression. This observation provides further evidence that basal-like cancers represent a heterogeneous group. In addition, it raises the possibility that targeting the AR pathway may represent a novel therapeutic approach to the management of patients with basal-like cancers, a group for whom the identification of therapeutic targets is an important clinical goal.

142 Androgen Receptor (AR) Expression in Ductal Carcinoma In Situ (DCIS) and Its Relationship to Molecular Subtype

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Background: Distinct subsets of invasive breast cancer have been identified by their patterns of gene expression and include luminal (A and B), HER2 and basal-like types. Recent studies have also identified the same molecular subtypes in DCIS, albeit with different frequencies in invasive cancers. Prior studies have demonstrated that AR is expressed in many invasive breast cancers and in DCIS. However, the frequency of AR expression in relation to the subtypes of DCIS defined by molecular analysis has not been previously studied in detail.

Design: We constructed tissue microarrays (TMAs) from paraffin blocks of 3,093 breast cancers that developed in women enrolled in the Nurses' Health Study between 1976-1996. TMA sections were immunostained for ER, PR, HER2, CK5/6 and EGFR. Results of these stains were used to categorize invasive cancers and DCIS as luminal A (ER+ and/or PR+ and HER2-); luminal B (ER+ and/or PR+ and HER2+); HER2 (ER- and PR- and HER2+); basal-like (ER-, PR-, HER2- and EGFR or CK5/6+); or unclassified (negative for all markers). TMA sections were also immunostained with a monoclonal antibody to AR. The relationships between AR expression and molecular subtypes of DCIS were analyzed.

Results: There were 228 DCIS with complete immunophenotypic data: 65% were luminal A, 13% luminal B, 13% HER2, 7% basal-like and 2% unclassified. Overall, 85% of DCIS cases were AR-positive but the frequency of AR expression differed significantly across the molecular phenotypes (p=0.002). AR expression was commonly observed in DCIS with luminal A and luminal B phenotypes (91% and 83% of cases, respectively), but was less frequently seen in the HER2 subtype (77% of cases). Moreover, despite their being defined by absence of ER and PR expression, 69% of basal-like DCIS showed expression of AR.

Conclusions: Among DCIS cases, AR expression is most often seen in luminal A and B phenotypes. However, expression of AR is also seen in approximately two-thirds of basal-like DCIS, despite the absence of ER and PR expression. This observation raises the possibility that targeting the AR pathway may represent a novel approach to the prevention of breast cancer in patients at increased risk for the development of basal-like breast cancers.

143 Outcome of Women with Ductal Carcinoma In Situ (DCIS) Treated with Breast-Conserving Surgery Alone: A Case-Control Study of 225 Patients from the Cancer Research Network

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Background: Radiation therapy (RT) following breast-conserving surgery (BCS) is now the standard treatment for most women with DCIS. However, the identification of a subset of patients who can safely be spared RT and be adequately treated by BCS alone is an important clinical goal.

Design: Among 3,037 women with DCIS treated with breast-conserving therapy between 1990-2001 at three health maintenance organizations in the Cancer Research Network, 1,298 received BCS alone. Within this group, we performed a case-control study of 225 women (97 cases;128 controls) to assess clinical and pathologic factors associated with local recurrence (LR). Histologic sections were reviewed by pathologists blinded to case-control status. Immunostains for various biomarkers were performed on paraffin sections. Adjusted relative risks (RRs) for LR were calculated for clinical and pathologic factors.

Results: Among the women treated with BCS alone, the 5-yr cumulative LR rate was 16%. Detection of DCIS as a mass or other clinical symptom was associated with an

increased risk of LR (RR=2.4; 95% CI 1.1-5.6). Among the pathologic features analyzed, LR was not significantly related to nuclear grade, architectural pattern, or comedo necrosis. Patients with a single low power field (LPF) of DCIS had a significantly lower LR risk compared to those with ≥10 LPFs (RR=0.3; 95% CI 0.1-0.9). In addition, those with positive or uncertain surgical margins had over a three-fold increase in LR risk compared to those with negative margins (RR=3.6; 95% CI 1.4-9.4). Close margins (<1 mm) were associated with an increased LR risk of borderline significance (RR=2.6; 95% CI 1.0-6.6). None of the biomarkers studied (ER, PR, HER2, Ki67, VEGF, p53 and COX2) were significantly related to risk of LR. In addition, using combinations of ER, PR and HER2 status as surrgoates for molecular phenotype (luminal A or B, HER2 and basal types), we found no signifcant associations between molecular subtype of DCIS and risk of LR.

Conclusions: Among this population of women with DCIS treated with BCS alone the LR rate was unacceptably high (~3%/year). The only pathologic features significantly related to LR were larger lesion size and positive or uncertain margins of excision.

144 Do Clinical and Pathologic Features of Ductal Carcinoma In Situ (DCIS) Vary with Patient Age? An Analysis of 657 Patients
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Background: Prior studies have indicated that young age at diagnosis is associated with an increased rate of local recurrence (LR) among women with DCIS treated with breast-conserving therapy. However, whether this can be explained by differences in clinical or pathologic features of DCIS according to age has not been examined in detail.
Design: Among 3,037 women with DCIS treated with breast-conserving therapy between 1990-2001 at three health maintenance organizations in the Cancer Research Network, 657 were enrolled in a case-control study to assess clinical and pathologic factors associated with LR and had histologic sections available for review. In this analysis, we compared clinical and pathologic features of DCIS in women across four age groups: <45 yrs (n=111), 45-54 yrs (n=191), 55-64 yrs (n=160), and 65+ yrs (n=195).
Results: DCIS presented as a mammographic abnormality less often in younger than in older women (68% for women <45 yrs; 82% for those 45-54 yrs; 81% for those 55-64 yrs; 86% for those 65+ yrs) (p=0.003). Among the pathologic features analyzed, DCIS extent as determined by the number of low power fields (LPF) of DCIS was greater in younger than in older women (mean #LPF 18.6, 14.2, 10.8 and 11.3 for women <45, 45-54, 55-64 and 65+ yrs, respectively; p<0.001). In addition, cancerization of lobules was present more often in younger than in older women (77% for women <45 yrs; 73% for those 45-54 yrs; 66% for those 55-64 yrs; 50% for those 65+ yrs) (p<0.0001). Of note, neither architectural pattern, nuclear grade, nor comedo necrosis varied significantly according to age. Furthermore, there were no significant differences in the frequency of expression of ER, PR or HER2 by age. Using the combination of ER, PR and HER2 status as surrogates for molecular phenotype to categorize DCIS lesions as luminal A, luminal B, HER2 and basal-like types, we found no statistically significant association between age and molecular subtype of DCIS.
Conclusions: In women <45 yrs of age, DCIS more often presents with symptomatic disease, is more extensive, and more often shows cancerization of lobules than DCIS in older women. Whether these features explain in part the higher LR rate in young women with DCIS treated with breast conserving therapy requires further study.

145 Expression of Cartilage-Specific Markers in Matrix-Producing Carcinoma of the Breast
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Background: Matrix-producing carcinoma (MPC) is a rare variant of metaplastic breast carcinoma with mixed epithelial and myxochondroid mesenchymal elements. Previous studies indicated that MPC are of epithelial origin and are associated with a myoepithelial-like differentiation. Little is known about the precise matrix composition or if a true chondrogenic transition occurs. The differentiation of mesenchymal cells into chondrocytes involves a multistep pathway and can be traced by evaluating marker genes of chondrocytic development. The transcription factor SOX9 is expressed early in mesenchymal condensation and has, with the chondroitin sulfate proteoglycan versican, an essential function in the subsequent differentiation of prechondrogenic precursor cells into chondrocytes. Differentiated chondrocytes express cartilage-specific markers such as the chondroitin sulfate proteoglycan aggrecan.
Design: Archival paraffin embedded material of 11 MPC as defined by the 2003 WHO classification and 2 axillary lymph node metastasis, one with a cartilaginous matrix component and one without, were examined by IHC for the expression and localization of SOX9, versican, aggrecan, p63 and S100.
Results: Tumor cells within the matrix revealed moderate to strong nuclear staining for p63 (13/13) and nuclear and cytoplasmic staining for S100 (13/13). Strong SOX9 nuclear expression was observed in the metaplastic carcinoma cells (11/11), and moderate SOX9 expression in the adjacent conventional carcinoma and in both lymph node metastasis. Strong staining for versican was observed in a cytoplasmatic pattern in the metaplastic tumor cells as well as the adjacent invasive conventional carcinoma. All 11 cases showed variable degrees of immunoreactivity for aggrecan. None of the cartilage biomarkers were expressed in normal mammary tissue.
Conclusions: MPC display cartilaginous differentiation and express cartilage specific matrix molecules. Our findings support that breast carcinoma cells of initially epithelial origin transdifferentiate into chondrocyte-like cells following the pathway of chondrocytic development. Thus, the results of this suggest that cartilage formation in MPC is a result of (myo)epithelial to mesenchymal transition.

146 Outcome of Papillary Carcinoma of Breast, One Institutional Experience
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Background: Papillary carcinomas of breast are uncommon; there are limited data regarding pathologic characteristics and outcomes. It has also been noted that distinguishing among intracystic papillary carcinoma, intracystic papillary carcinoma with invasion and invasive papillary carcinoma can be challenging. The aim of this study is to describe natural history of papillary carcinoma and its long-term prognosis.
Design: The cases with diagnoses of intracystic papillary carcinoma with or without invasion, invasive papillay carcinoma including solid papillary carcinoma variant were included in this study. The cases were retrieved through a search of the departmental database in the period of 1990-2006. The medical records were retrospectively reviewed with regard to clinical history, pathologic finding, treatment approach and outcomes (mean 7.1 years follow-up).
Results: The study involved total of 25 women with an average of 63 (41-84) years old. Eighteen patients had pure papillary carcinoma and 10 had invasion. Seven patients had papillary carcinoma with coexisting in-situ and/or invasive ductal or lobular carcinoma with three of them having invasive papillary carcinoma. Tumor size measured from 0.5 to 4.2 cm. ER was positive in 86.7%. Her-2/neu was tested in 8 patients and all were negative. Triple-negative receptor status was observed in one patient. Treatment involved combination of surgery, tamoxifen and radiation, as well as surgery and/or chemotherapy and radiation. Nodal status was assessed in 10 patients and metastasis was seen in two patients with pure invasive papillary carcinoma. These two patients were tumor-free for 3 and 18 years, respectively. Distant metastasis was observed in only one case, at year six of follow-up in a patient with nodal negative mixed papillary ductal carcinoma.
Conclusions: Although a small-scale study, the above data support the theory that papillary breast carcinomas has an indolent natural history and should be distinguished from tumors with ductal phenotype.

147 Benign Breast Biopsies in Women That Later Developed Carcinoma: Distinctive Histologic Features and Stem Cell Marker Expression
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Background: Identification of the 10% of women undergoing a breast biopsy with a benign diagnosis who develop breast cancer in the future is important for prevention. The characterization of histological features and predictive biomarkers in these patients are not well-established. We evaluated the incidence and type of proliferative lesions and characterized the expression of two novel stem cell markers, ALDH1 and EZH2, in benign breast biopsies of women who later developed breast cancer.
Design: Women who had biopsies of benign breast lesions between 1970 and 2002 and subsequently developed breast cancer were studied histologically. The diagnosis of atypical ductal hyperplasia (ADH) and flat epithelial atypia (FEA) were made following strict published criteria. Immunohistochemistry was performed for EZH2 and ALDH1. The percentage of EZH2 expressing cells was recorded. ALDH1 was evaluated as positive or negative. Benign breast tissues from women who did not developed carcinoma served as controls.
Results: Twenty-four benign breast biopsies were available. The majority of the biopsies had proliferative lesions including prominent and tumoral adenosis (33%, 8/24), papillomas (12.5%, 3/24), florid intraductal hyperplasia (25%, 6/24), and epithelial atypias (ADH and FEA, 29%, 7/24), which exceed those reported for benign biopsies in the general population. Fifty % of the biopsies contained reactive cells with enlarged nuclei and prominent nucleoli with frequent mitoses. Twenty of 24 benign biopsies were available for immunohistochemistry. EZH2 and ALDH1 positive cells were present in 95% and 45% of the biopsies, when compared to 10% and 5% of the control biopsies, respectively. The mean percentage of EZH2 positive cells was 30% when compared to 5% in the control group. The highest EZH2 expression was noted in the reactive cells and papillomas (up to 90% and 80% of EZH2 positive cells, respectively).
Conclusions: Benign breast biopsies from women who later developed breast cancer exhibit frequent reactive cells with large nuclei, papillary lesions, and tumoral adenosis. ADH and/or FEA are also more common in these biopsies when compared to benign breast biopsies in the general population. We characterized for the first time the expression of EZH2 and ALDH1 stem cell markers and showed that both markers are upregulated in high risk proliferative breast lesions. We are currently increasing the number of cases to define if EZH2 and ALDH-1 can reliably identify women at risk of developing breast cancer.

148 Pathologic Response and Microvascular Surface Area in Breast Cancer Treated with Neoadjuvant Chemotherapy
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Background: Breast cancer may be treated with neoadjuvant chemotherapy (NACT) to reduce locally advanced disease and evaluate response to treatment. Chemotherapy-induced response ranges from complete to absent, and is assessed histologically after excision. Vascular parameters such as microvessel density (MVD) and microvascular surface area (MVSA) may be associated with the tissue response to chemotherapeutic agents, but their role is not well defined.
Design: 62 patients enrolled in a phase II clinical trial for sequential NACT with doxorubicin (A) and paclitaxel (T) were randomized into two groups (A/T and T/A). H&E sections of tumors were examined from all patients before, during and after treatment. Pathologic response evaluation included size, cellularity, viability, histologic type and grade of residual carcinoma, and lymph node metastases. Tumors were assessed following NSABP-B18, Miller-Payne Grade system (MPG), Modified Nottingham Prognostic Index (MNPI), and Sataloff post-treatment pathologic evaluation. Immunohistochemical staining for the vascular marker CD31 was done on

10 representative samples (5 from each group). MVD was calculated at magnification x200, and MVSA [μ²] determined by Image J software.
Results: Tumor size decreased from 3 (2.8) cm mean (median) before treatment, to 1.9 (1.5) cm after treatment. NSABP-B18 pathological response was complete in 9%, partial in 68% and none in 23%. 39% of cases showed 30-90% tumor cell reduction (MPG-3), 19% no reduction (MPG-1) and 7% no invasive tumor cells (MPG-5). Lymph nodes were negative in 49% (Sataloff N-A, N-B); nodal metastases with (N-C) and without treatment effect (N-D) were present in 21% and 30%. 96% of residual tumors appeared viable; 38% showed mitoses. MNPI was most favorable for 57%. MVSA decreased from 253 to 87μ² in group A/T, and increased from 50 to 145μ² in group T/A. Average MVD was 13 and 16/field before NACT, 17 and 19/field after NACT in groups A/T and T/A, respectively.
Conclusions: This morphometric study shows MVSA to be variable in sequential chemotherapy regimens with paclitaxel and doxorubicin. This finding contrasts with stable MVD and overall decrease of tumor cellularity. While it is still unclear how certain chemotherapeutic drugs may influence vascular patency and possibly drug delivery, this study supports that vascular dynamics and pathological response both are important factors in the effects of chemotherapy.

149 A 19-Gene Classifier Distinguishes Invasive Ductal and Classic Lobular Breast Carcinomas: Amplification of Chromosome 8q Characterizes Ductal While Estrogen-Related EGR-1 Characterizes Lobular Cancers
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Background: Invasive ductal and lobular breast carcinomas (IDCs and ILCs) have distinct clinico-pathologic profile, spread and response to chemotherapy. Cases of 'mixed ductal and lobular histology' (IDLCs) are not uncommon. Aim of this study was to decipher the molecular differences between histologically pure IDCs and ILCs and to develop a classifier that could further define the molecular basis of IDLCs.
Design: Fresh frozen IDC-ILC tissues (n=76) from 64 adult female patients with primary breast cancer were subjected to gene expression profiling on Affymetrix u133 plus 2.0 GeneChip arrays. Archival sections were reviewed to select pure ductal (N=49) and pure lobular, classic-subtype (N=27) (excluding basal-like IDCs) for analysis. Data were normalized using robust multichip averaging and post-processed with ComBat. A 115-fold nested cross validation was used to obtain an optimal support vector machine gene classifier that distinguished IDCs and ILCs. The performance of these classifiers had a median accuracy of 89%, with a 93% sensitivity and 78% specificity. The genes that appeared most frequently (>60%) across classifiers were selected to provide a robust gene signature.
Results: Based on supervised analysis of gene expression profiles of 'histologically pure' IDCs and ILCs a 23 probeset, 19-gene classifier was developed. Our classifier could accurately cluster pure IDCs and ILCs into 2 distinct molecular subgroups, except 2 of 27 ILCs clustered with the IDCs (ductal like ILCs). Prominent genes in this classifier included CDH1 and EGR1 which were down regulated and up-regulated in ILC respectively. In addition, 7/19 genes were expressed and predictive of IDC co-localized to chromosome 8q21-24 indicative of amplification of this locus. We successfully cross-validated our 19-gene classifier on publically available IDCs-ILC gene expression data sets.
Conclusions: Our 19-gene classifier supports that pure IDCs and ILCs are distinct molecular subtypes, and suggest they have distinct chromosomal abnormalities. Based on this classifier, the mixed ductal-lobular histology cases may be assigned to pure ductal cancers or may be truly mixed to merit further investigation.

150 Predictive Markers (ER, PR, HER2, HER2FISH) Pre- and Post-Neoadjuvant Chemotherapy in Primary Breast Carcinoma
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Background: The estrogen receptor (ER), progesterone receptor (PR), and HER2 profile of a female primary breast carcinoma plays a significant role as a predictive marker in patient management and treatment. Because of the increasing utilization of neoadjuvant chemotherapy or hormone therapy, surgically resected carcinomas often show marked treatment effect. The aim of this study was to compare immunohistochemical (IHC) profiles (ER, PR, HER2, HER2 FISH) of primary breast carcinomas before and after neoadjuvant chemotherapy to assess the effects of these treatment modalities on hormone receptor status.
Design: Primary breast carcinomas from 55 female patients treated with neoadjuvant therapy after needle core biopsy or fine needle aspiration diagnosis between 2004-2008 were included. Histologic data was collected for each case, including site, type, grade, tumor size (cm), pre- and post- neoadjuvant treatment IHC panel (ER, PR, HER2), and fluorescence in-situ hybridization (FISH) for HER2. Clinical information (stage, treatment type, and lymph node status) was obtained. Exclusion criteria included patients not treated with neoadjuvant chemotherapy or cases with incomplete IHC data, resulting in a final sample size of 38.
Results: Of the 38 carcinomas studied, 53% were grade III, 92% were of ductal type, and 58% had metastasized to axillary lymph nodes. The mean size of carcinomas was 3.9 cm (range 0.1- 12 cm). Forty-five percent were positive for ER by IHC both pre- and post- neoadjuvant treatment (P=1.00). IHC studies for PR in these 38 patients showed 37% positivity for PR pre-neoadjuvant therapy and 21% positivity post-treatment, with a statistically significant P value of 0.03. For 37 patients with HER2 IHC, 32% were positive pre-treatment, and 22% were positive post-treatment (P = 0.20). For 7 patients, HER2 FISH was positive in 71% pre-therapy and in 57% post-treatment (P=0.32). Overall concordance ranged from 73.0-89.5%.

Conclusions: Profiles for ER, HER2 IHC, and HER2 FISH were not significantly different in primary breast carcinomas before and after neoadjuvant chemotherapy. There was a statistically significant decrease in PR positivity post-treatment. Further investigation is warranted to assess reproducibility of technique and investigate clinical implications of loss of PR status in treated patients.

151 Making Sense of the Her-2/neu (HER2) Immunohistochemistry (IHC) Equivocal Category Post-SCO/CAP Guidelines
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Background: ASCO/CAP recently developed guidelines for HER2 testing and reporting to improve its accuracy as a predictive marker. A new equivocal IHC category was created defined as circumferential but weak membrane staining in more than 10% of cells or strong membrane staining in less than 30% of neoplastic cells. Tumors in this category are poorly defined and have uncertain benefit from anti-HER2 therapy. This study was designed to determine the characteristics of this group in comparison to the IHC positive (+) and negative (-) cases and to determine its significance as a prognostic and predictive marker.
Design: 825 consecutive cases of invasive breast carcinomas were selected from our files over a 2 year period. The HER2 IHC was performed using PathwayHer2 antibody fromVentana using Benchmark automated stainer and reported using the Ariol Scan system by Applied Imaging Corp. All the cases were also reviewed independently by two pathologists and the HER2 staining intensity and percent distribution was evaluated manually. HER2 FISH analysis was performed by the PathVysion kit and read manually. Results of IHC were correlated with the tumor characteristics, ER, PR status and HER2 FISH ratio.
Results: HER2 IHC+ carcinomas had more aggressive features as compared to the HER2 IHC- or the IHC equivocal group. Tumor characteristics of the IHC equivocal group were similar to that of the IHC- group. Chromosome 17 polysomy was more frequently observed in the IHC+ cases (21%). Further subdivision of the IHC equivocal group into FISH+ and – cases confirms the resemblance of FISH- to the IHC- and FISH+ to the IHC+ group.

Comparison of HER2 IHC positive, negative and equivocal cases.

	Total cases	Grade 3 %	Mean Ki67 %	ER+ %	PR+ %	FISH+ %	Polysomy %
IHC+	136	80.9	37.8+/-18.7	54.4	41.2	95.6	21.3
IHC-	99	38.4	29.3+/-24.3	79.8	77.8	10.1	8.1
IHC equivocal	128	36.7	27.7+/-23.3	88.3	71.9	21.1	9.4
IHC equivocal FISH+*	27	62.9	42.1+/-24.7	74.1	59.3	NA	25.9
IHC equivocal FISH-*	94	26.6	22.9+/-21.3	93.6	75.5	NA	5.3

NA: Not applicable;* there were 7 FISH equivocal cases
Conclusions: Our study confirms the distinct phenotype of HER2+ breast carcinomas (high grade carcinomas, ER/PR-, high Ki67) as compared to HER2- carcinomas (low grade, ER/PR+, low Ki67). In the HER2 IHC equivocal group, carcinomas that are FISH+ show features similar to the HER2+ group, whereas the FISH- carcinomas show features of the HER2- group. Thus treatment of the IHC equivocal carcinomas should be based on FISH results.

152 Expression of CK5 Better Correlates with Shorter Survival and Poor Prognosis in Triple-Negative Breast Cancer
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Background: Triple-negative breast cancer (ER, PR, and HER2-negative) is a high risk breast cancer that lacks the benefit of specific therapy that targets these proteins. There is a great deal of overlap between triple-negative and basal-like breast cancers (BLC), but this overlap is by no means complete. The aim of our study was to investigate basal phenotype in a series of triple-negative invasive mammary carcinomas; to assess the expression of different biomarkers associated with basal phenotype; and to evaluate their relationship with prognosis.
Design: We selected 140 previously tested triple-negative tumors. Clinical, histopathological and survival data were obtained. A TMA containing 2 cylinders from each tumor was constructed and immunohistochemistry for ER, PR, HER2, CK5, CK14, EGFR, p63, caveolin, and p53 was performed. We considered BLC to be any tumor ER/PR/HER2-negative, CK5- and/or CK14-positive.
Results: We found 105 cases of BLC among the 140 triple-negative tumors (frequency=75.0%). The mean age at diagnosis was 54.8 years-old and 34.3% were premenopausal women. The majority of tumors were high grade (83.7%) and of ductal/no-special-type (80.8%). Triple-negative tumors showed immunoreactivity for CK5 (75.0%), CK14 (29.0%), EGFR (28.6%), p63 (28.6%), caveolin (14.3%), and p53 (67.1%). CK14 expression always coexisted with CK5 expression. Tumor size larger than 5cm was observed in 41 cases (39.0%) and axillary metastases were detected in 61 patients (59.2%). Follow-up was obtained for 89 patients (mean=51 months): 45 patients (50.5%) with no evidence of disease; 6 patients (6.7%) were alive with disease; and 38 patients (42.6%) died of the disease. Relapse was detected in 42 women (47.1%), lungs, brain, and bones being the most commom sites of metastases. The mean overall survival (OS) was 36 months and the mean disease-free interval was 28 months. OS in patients with triple-negative CK5-positive/basal tumors was shorter than OS in those with triple-negative CK5-negative/non basal tumors (RR=2.4). In contrast, positivity for CK14, EGFR, p63, or caveolin was not associated with poorer outcome.
Conclusions: Our findings confirmed that BLC have a poor prognosis and are high-frequent carcinomas among triple-negative tumors. Compared to other basal markers, CK5 expression was the most prevalent and the only one that improved recognition of patients with shorter survival in triple-negative breast cancer.

153 Anaplastic Large Cell Lymphoma Arising in Association with Breast Implants and Mimicking Recurrent Breast Carcinoma: A Report of Five Cases
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Background: Breast augmentation is the most commonly performed cosmetic surgery in USA. Cancer can rarely occur in fibrous capsules surrounding implants and carcinoma is the primary concern. Primary anaplastic large cell lymphoma (ALCL) of the breast occurring in association with breast implants is another rare entity to consider in this setting with only 12 cases documented in literature. We present an additional five cases of breast implant-associated ALCL.
Design: Five cases of primary ALCL of breast associated with implants were retrieved from files of William Beaumont Hospital (2 cases), MD Anderson Cancer Center (2 cases) and Consultatoria em Patologia, Brazil (1 case). Clinical and pathologic information was obtained.
Results: The patient ages ranged from 28 to 63 years. Breast augmentation was performed for cosmetic reasons in 2 cases and reconstruction following cancer surgery in 3 cases. Two patients had saline-filled implants, two had silicone gel-filled implants and one pateint had saline filled implant with replacement by silicone gel-filled implant. Time of implant to diagnosis of ALCL ranged from 6 to 10 years. Two patients presented with implant-related contracture, two with peri-implant edema and the fifth case presented with hematoma following trauma. All cases showed anaplastic large dyscohesive tumor cells within the fibrous capsule of implant with associated extensive necrosis. The tumor cells stained positive for CD30 and were negative for ALK-1. Four were of T-cell lineage while one case was of null-cell type. Three patients received chemotherapy and were alive and disease free (4-10 years). One patient has insufficient follow-up due to recent diagnosis and one patient was lost to follow-up.
Conclusions: Pathologists need to be aware of this rare neoplasm which may occur in association with breast implants. These cases highlight the importance of examining breast capsule specimens. Presence of malignancy should prompt submitting the rest of the capsular tissue for evaluation, performing appropriate immunostains especially CD30, and obtaining a hematopathology consult if required. Implants whether saline- or gel-filled generally have silicone elastomer shells and the occurrence of ALCL in association with breast implants suggests a possible etiopathogenetic relationship.

154 The Novel Estrogen-Induced Gene EIG121 Is Up-Regulated in Estrogen Receptor Positive Breast Cancer, Significantly Associated with Recurrence Free and Overall Survival, and Is Associated with Sensitivity to Tamoxifen
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Background: The development of endocrine resistance in estrogen receptor (ER) positive breast cancers represents a significant obstacle in the clinical managment of breast cancer patients. The mechanisms underlying such resistance, however, are unknown. 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 from non-estrogen dependent non-endometrioid endometrial carcinoma. Because of these interesting expression characteristics in endometrial cancer, we hypothesized that EIG121 would be tightly linked to ER positive breast cancer and may play a role in determining hormone responsiveness.
Design: EIG121 expression was quantified using qRT-PCR in 30 normal and breast cancer tissues and by applying reverse phase protein lysate array (RPPA) in 460 breast cancers. EIG121 expression was also analyzed in tamoxifen-sensitive and -resistant MCF7 breast cancer cells. A tetracycline-inducible cell system was generated to analyze the effect of EIG121 in EGFR-mediated cell signaling, as this pathway has been hypothesized to mediate endocrine resistance.
Results: EIG121 was strongly over-expressed in ER positive breast cancers compared to normal breast tissue and ER negative cancers. In multivariate analysis of the RPPA data, high EIG121expression was a significant predictor of both recurrence free survival and overall survival. In 258 of the patients with ER positive cancers treated with adjuvant tamoxifen, high levels of EIG121 were significantly associated with recurrence free survival (p=0.0364). EIG121 was over-expressed in tamoxifen-sensitive breast cancer cells, but not detected in tamoxifen-resistant cells. EIG121 binds to EGFR, promoting its degradation and inhibiting downstream signaling via Akt.
Conclusions: The expression characteristics of EIG121 in breast cancer suggest that it is strongly associated with factors important in survival. Furthermore, our experiments in cell-based systems suggest that EIG121 plays a direct role in determining a breast cancer cell's sensitivity to endocrine-based therapy.

155 Estrogen Receptor (ER) Expression in Breast Carcinoma: Comparison of Antibodies (Ab) SP1 and 6F11.
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Background: ER status assessment is critical for the optimal management of breast cancer (BrC) patients. We compared the new SP1 rabbit monoclonal Antibody (Ab) with Ab 6F11, which had been validated in our institution, through clinical trials, Q/A programs and comparison with biochemical assay values.
Design: Following the manufacturers' instructions, we stained with SP1 and 6F11 quadruplicate TMAs of 115 consecutive invasive BrC. Also SP1 was applied to 511 randomly selected whole sections from BrC samples (mostly invasive BrC in excision specimens) for which the orignal 6F11 IHC slides were reviewed and both were classified according to their ER status: negative (score 0, no or less than 1% cells

with mild staining); positive, score 1 (less than 10% cells stained), and 2 (more than 10%), with a separate evaluation of staining intensity and background staining. Slides from 7 non-breast benign tissue samples were also stained in parallel with both Abs. All borderline (including all 0 and 1) results were reviewed and confirmed by two pathologists (JD, TH, or SF).
Results: All 115 TMA cases had the same ER expression pattern with both Abs (81 positive, 19 negative). 4/110 6F11-negative cores were positive with SP1, and 6F11 was positive in 1/107 SP1-negative cores. Of the 511 reviewed whole sections, concordance between SP1 and 6F11 was noted in 97.04 % of cases (kappa=0.90). Most (99.5 %, 2/414) of 6F11 positive cases were also positive with SP1. Also, 13/97 (13.4 %) cases scored negative by 6F11 were found to be positive by SP1 (Fisher's p=0.0035). 12 of these 13 cases had a score of 1. Significant differences in staining within the same score occured in 18.6 % of cases (n=95), and favored either SP1 (n=59) or 6F11 (n=36). Signal to noise ratio was described as better in SP1 (n=40), or 6F11-stained slides (n=11) or similar (rest), although SP1 generally tended to be "crisper". In benign tissues, expected normal staining patterns were generally more obvious with SP1; however, enhanced staining of stromal cells and lymphocytes related to this Ab was seen as a real potential pitfall, also noted in BrC cases.
Conclusions: SP1 and 6F11 are comparable, but in BrC samples, SP1 provides slightly increased sensitivity and equal or better signal to noise ratio. SP1 will strongly stain stromal cells and lymphocytes in BrC samples, a pitfall to be aware of when assessing ER status in breast carcinoma samples. Most discordant SP1+/6F11 neg. cases have less than 10 % of cells stained with SP1.

156 Basal-Like Ductal Carcinoma In Situ: Immunohistochemical and Morphologic Characterization
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Background: Recent gene expression profiling studies have identified a distinct basal-like breast carcinoma. The basal-like carcinoma is typically estrogen receptor (ER)/progesterone receptor (PR) and HER2 negative (triple negative), expresses basal keratins (CK5/6, CK14), and is positive for epidermal growth factor receptor (EGFR). Most significantly, this group of tumors is associated with the highest proliferation rates and the poorest clinical outcome and has been described as the prevalent subtype in BRCA1 related breast carcinomas. The precursor histologic and molecular pathway leading to basal-like carcinoma is poorly understood. The aim of this study is to characterize the immunohistochemical and morphologic profile of in situ basal-like carcinoma and contrast this with its invasive counter-part.
Design: The following triple negative cases were evaluated: 28 cases of high-grade ductal carcinoma (HGDCIS) with associated invasive ductal carcinoma (IDC) and 13 pure HGDCIS. Their pathologic features were reviewed. Biomarker expression was examined using immunostaining for CK 5/6, CK14, EGFR, Ki67, p16, Cox2, and BRCA1.
Results: Eighteen of the 28 cases (64%) of triple negative HGDCIS with associated IDC were classified as basal-like based on positive immunostaining for CK5/6, CK14, and/or EGFR. The majority of these cases (93%) had a Ki67 index >10%, 81% showed strong positive immunostaining for p16, 76% were Cox2+, and 82% were BRCA1+. The in situ and invasive components of all cases showed similar immunophenotype. Of the 13 cases of pure HGDCIS, 9 (69%) were classified as basal-like. Only 5 cases (55%) had a Ki67 index >10%, however a similar percentage of cases showed strong positive staining for p16 (67%), were Cox2+ (78%), and were BRCA1+ (75%). Most triple negative HGDCIS, regardless of basal phenotype shows solid and comedo architectural patterns (80%) and is associated with prominent periductal inflammation (60%).
Conclusions: Overall, basal-like HGDCIS and IDC show similar immunohistochemical profiles. Both demonstrate positive staining for p16 and Cox2 in the majority of cases, supporting recent reports that overexpression of p16 is characteristic of the basal-like subtype. In addition, most cases of HGDCIS and HGDCIS with IDC were BRCA1+, suggesting that while basal-like tumors are the most prevalent subtype in BRCA1 related breast carcinomas, the converse is not true. Finally, we saw a subtle but significant difference in the Ki67 index between pure HGDCIS and HGDCIS associated with IDC.

157 Simulations of Growth of DCIS Parameterized from IHC
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Background: Few models describe the growth of DCIS in duct spaces in three dimensions. It would be useful to understand the natural history of DCIS, its potential for progression to invasive carcinoma (IC), and whether the model can improve upon imaging studies to predict the size of a tumor from biopsy material prior to surgery.
Design: We use immunohistochemistry (IHC) results from fixed tissue to measure proliferation and apoptotic indices as input to a multiscale model of tumor growth to predict the growth curves for DCIS. Extension by pagetoid spread and expansion of ducts is not included in this model. Growth within an avascular duct space, effects of nutrient and oxygen access on proliferation, and necrosis is accounted for. Our preliminary results include 13 case of solid, mixed solid-cribriform, and cribriform type DCIS. Cases with co-existing IC were excluded. The cases were selected to provide a set of small (< 5 cm) and large (> 5 cm) tumors regardless of grade. We predict time to reach 95% of maximum size and geometric mean of tumor dimensions and compare these with sizes predicted from mammography and measured from pathology.
Results: Rate of apoptosis is low and best represented by a constant value across all cases, while rate of proliferation varies. Predicted results show good agreement with the geometric mean of the size measured from pathology. Model results are closer to the actual size for solid and mixed solid-cribriform tumors than mammographic estimates, and indicate that there is an initial fast rate of growth followed by a very slow rate of growth for DCIS.

Conclusions: Multiscale modeling methods may be able to predict size of DCIS better than imaging if adequate material can be obtained to estimate input parameters. Results suggest that increased ability to proliferate may be an early step in development of DCIS. We hypothesize that most cases of DCIS have reached their maximum size by the time they are diagnosed. We raise questions about how rapidly DCIS might evolve to IC once it has reached maximum size given the number of cases of IC diagnosed with co-existing DCIS compared to number of cases of DCIS diagnosed without invasion (ratio > 2:1). We note the effective use of ex vivo tissue instead of in vitro systems to measure input for computer simulations.

158 Significance of Focal Lobular Neoplasia in Breast Core Needle Biopsy
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Background: Management of Lobular neoplasia (LN), encompassing atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS) remains controversial. Many studies suggest surgical excision following a diagnosis of LN on core needle biopsy; others see no need for any intervention. This study is aimed to assess the risk for concurrent invasive carcinoma or ductal carcinoma in situ.
Design: We searched the database of the Pathology Department at a tertiary referral center for breast core biopsies from July 1997 to June 2008. We identified all biopsies diagnosed as LN, LCIS, and ALH. All biopsies with concurrent diagnosis of atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS), pleomorphic variant of LCIS, or invasive carcinomas were excluded from the study. Immunostain for E-cadherin was performed on all cases. Follow-up studies were reviewed. The presence of flat epithelial atypia (FEA) was also evaluated.
Results: 3763 breast biopsies were performed during this period. 176 patients (4.6%) were diagnosed with LN. 127 patients were excluded from this study due to the presence of other malignant or premalignant lesions. 49 cases (1.3%) were diagnosed with LN or LN with a benign lesion. 10 patients were excluded due to lack of follow-up. Of the remaining 39 patients; 4 had prior history of invasive cancer and 9 had a family history of breast or ovarian cancer. Lobular neoplasia was confirmed by E-cadherin on all but 6 core biopsies in which the area of interest was absent on the deeper cut. 25 of these patients had biopsies due to abnormal calcification. 8 patients had associated mass lesions, and 6 patients had other radiographic abnormalities. 7 patients had associated FEA. The follow-up excision of 2 of the 39 patients (5.1%) showed DCIS. One of them had prior history of infiltrating carcinoma, and the other had a family history of breast cancer. A third patient had a 9 mm focus of infiltrating carcinoma on excision. This patient had also prior history of infiltrating lobular carcinoma in the same breast. The mammogram of all three patients showed mass lesion, one of them also showed calcifications. Only one of the three patients had FEA. 24 of 39 patients (61%) in this study had additional foci of LN on excision.
Conclusions: These results suggest the diagnosis of non-pleomorphic variant of LN on core needle biopsy may not mandate excision in all cases. Our study also suggests that FEA associated with LN probably does not carry any significant additional risk, however; more detailed studies on FEA are needed.

159 Predictive Markers in Primary Breast Cancer Compared with Lymph Node and Bloodspread Metastases
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Background: Breast cancer is the most common female cancer in the US, and the main cause of death in women ages 45 to 55. Women with hormone receptor-positive tumors benefit from postoperative Tamoxifen or Aromatase Inhibitors. High levels of HER2 expression identify those patients who might benefit from treatments that target HER2. Among women with metastatic breast cancer, the predictive markers may be different from the primary tumor. We compared predictive markers: Estrogen Receptor (ER), Progesterone Receptor (PR) and HER2 of primary breast carcinomas with those of lymph node (LN) and blood spread metastases (BM).
Design: This study includes 66 women between the ages of 28 to 86 years. Forty-nine patients had LN metastases and twenty-eight patients had BM. ER, PR and HER2 were performed on available aspiration cell blocks on metastatic lesions and primary tumor core biopsies using FDA approved antibodies and HercepTest (Dako). ER and PR were positive when >/=10%. Her2 was positive (amplified/expressed) when 3+ >30% by immunostain or >2.2 by FISH.
Results:

Comparisons	N	Overall agreement %	Kappa	Positive, Breast	Positive, LN	P Value
Breast ER vs. LN ER	42	76.2	0.52	64%	55%	0.57
Breast PR vs. LN PR	42	73.8	0.48	48%	26%	0.06
Breast HER2 vs. LN HER2	39	59.0	0.28	8%	26%	0.07
Breast FISH vs. LN FISH	10	70.0	0.46	30%	30%	0.39
Comparisons	N	Overall agreement %	Kappa	Positive, Breast	Positive, BM	P Value
Breast ER vs. BM ER	19	68.4	0.30	79%	58%	0.45
Breast PR vs. BM PR	18	61.1	0.28	61%	33%	0.31
Breast HER2 vs. BM HER2	17	52.9	0.28	24%	24%	0.45
Breast FISH vs. BM FISH	4	75.0	0.50	50%	25%	0.32
Comparisons	N	Overall agreement %	Kappa	Positive, LN	Positive, BM	P Value
BM ER vs. LN ER	12	33.3	0.00	50%	33%	0.92
BM PR vs. LN PR	12	33.3	0.00	50%	17%	0.57
BM HER2 vs. LN HER2	11	36.4	0.00	9%	18%	0.57
BM FISH vs. LN FISH	1	-	-	-	-	-

Conclusions: All three predictive markers are similar in the primary and two metastatic sites (lymph node, bloodspread). Only in primary vs. lymph node metastasis is there a tendency for PR and HER2 (P values 0.06, 0.07) to be different. For HER2, the majority

of lymph node metastases are in cell blocks (FNA), fixed in ethanol rather than formalin, which may have caused false positive HER2 expression.

160 HER2/neu Testing in Breast Carcinoma. Concordance Study Fluorescent In Situ Hybridization (FISH) vs. Chromogenic In Situ Hybridization (CISH)
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Background: HER2/neu testing for breast cancer is primarily done in laboratories using two methodologies, i.e. immunohistochemistry (IHC) for identifying HER2/neu receptor protein over expression and fluorescent in situ hybridization (FISH) for HER2/neu gene amplification. With the recent development in technology, HER2/neu gene amplification can be detected by chromogenic in situ hybridization (CISH). FISH technology requires a fluorescence microscope and special skills and hence cannot be performed by many labs. CISH test slides can be scored using an ordinary light microscope and there is no loss of signals over a long period of time. Both of these are advantages over FISH. The objective of our study was to determine the concordance between FISH versus CISH HER2/neu testing in breast carcinoma.
Design: Fifty cases of invasive breast carcinoma from excisional biopsy specimens with known HER2/neu status by immunohistochemistry were identified from the pathology database. The IHC test scores (using DAKO A0485 antibody) of study group were as follows: 0 (24 cases), 1+ (4 cases), 2+ (9 cases), and 3+ (13 cases). HER2/neu gene amplification was determined by FISH (Vysis Pathvision) and by CISH (ZYMED Inc.). The FISH results were assessed as following: If HER2/CEP17 ratios > 2.2 amplified; <1.8 not amplified; and 1.8-2.2 equivocal. For CISH, the number of HER2 signals per nucleus were counted within 60 nuclei of invasive carcinoma cells, with <6 signals defined as not amplified and ≥6 signals as amplified. The scoring of CISH results was done by one investigator with no prior knowledge of FISH results. The FISH results were assessed by another investigator with no prior knowledge of CISH results.
Results: This study showed that 49/50 (98%) cases were concordant for FISH and CISH. 1/50 case was discordant (table 1). In this case, no signals were observed (tissue not optimal) by FISH but was negative by CISH.

Table 1

	CISH	FISH	Comment
Amplified	15	15	100% concordant
Not Amplified	35	34	1 case discordant*

*One case no signals were seen by FISH
Conclusions: These results indicate that CISH can be used as an alternative method of testing for HER2/neu gene amplification.

161 Reproducibility of Needle Core Biopsy Diagnosis of Breast Papillary Lesions
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Background: Information on reproducibility of breast papillary lesion core biopsy diagnosis is limited. As part of a study assessing accuracy of core biopsies to identify breast papillary lesions requiring surgical excision, we evaluated interobserver agreement for classifying papillary lesions on needle core biopsy using a diagnostic algorithm.
Design: We designed a diagnostic algorithm based on WHO classification of papillary lesions and Page criteria for ADH and DCIS. Seven categories, capturing potential diagnostic thresholds for surgical excision, were created: (1) benign papilloma with no ductal proliferation; benign papilloma with (2) ≤10%, (3) >10% - <50%, (4) ≥50% UDH; (5) papilloma with ADH; (6) papilloma with DCIS; and (7) papillary carcinoma. Two senior and 2 junior breast pathologists piloted the algorithm by together reviewing a training set of 25 breast papillary core biopsies. We subsequently searched the pathology database from 1997-2006 for needle core biopsy diagnoses of papillary breast lesions. We excluded cores with concurrent invasive carcinoma, or with ADH or DCIS outside of the papillary lesion. Study cases, consisting of slides stained with H&E and for smooth muscle myosin heavy chain, were circulated to the 4 pathologists, for independent assessment using the diagnostic algorithm.
Results: The study consisted of 164 cases. There was substantial agreement for the 7 categories, with weighted k (kappa statistic) ranging from 0.69 to 0.80. Agreement was almost perfect for papillary carcinoma (k=0.85), moderate for papilloma with no ductal cell proliferation (k=0.51), slight for papilloma with DCIS (k=0.14), and fair for all other categories (k=0.23 to 0.36). Agreement was substantial when diagnoses were dichotomized into (a) ADH, DCIS or papillary carcinoma present vs benign papilloma with or without UDH (k=0.72; (b) benign papillomas with up to 10% or 50% UDH vs all other categories (k=0.77, 0.77, respectively), and moderate when threshold was (c) papilloma with no ductal proliferation vs all other categories (k=0.52). There was unanimous agreement for category assignment in 75% of cases for (a), 79% for (b) and 67% for (c).
Conclusions: Using our algorithm, we were able to achieve substantial agreement on needle core biopsies for potential diagnostically relevant categories of papillary breast lesions among pathologists with varied experience in breast pathology.

162 Altered Histone Modifications and Their Regulating Enzymes in Fulvestrant Resistant Breast Cancer Cell Line *In Vitro*
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Background: Clinical studies have demonstrated that Fulvestrant is a valuable treatment in postmenopausal women with advanced breast cancer who have progressed on prior

endocrine therapy. However, Fulvestrant resistance commonly emerges, where *in vitro* data indicate this state can have increased proliferative and invasive capacity. Thus, understanding the mechanisms responsible for Fulvestrant resistance remains a key challenge if we are to improve treatment and survival for breast cancer patients. The purpose of this study was thus to begin to examine aspects of epigenetic regulation in a Fulvestrant resistant model, profiling histone acetylation/methylation and its modifying enzymes. Such studies could reveal new therapeutic avenues to treat endocrine-resistant breast cancer.

Design: A Fulvestrant-resistant breast cancer cell line (FASMCF), developed by continuous culture of the endocrine responsive MCF-7 parental line in Fulvestrant (10-7M)-supplemented medium. Immunocytochemistry was used to compare the level of a series of histone lysine acetylation (H3K18, H4K12, and H4K16), lysine methylation (H3K4, H4K20) and arginine methylation (H4R3) marks between the parental MCF7 and FASMCF cell lines prepared as paraffin cell pellets. Q-PCR was used to compare the level of various histone modifying enzyme genes (e.g. PCAF, NCOA1, HAT1, MYST3, HDAC1, HDAC2, and EZH2) in both cell lines.

Results: The level of histone lysine acetylation (H3K18, H4K16) and methylation (H4K20) was higher in FASMCF cells compared to MCF-7 cells, as revealed by nuclear immunostaining. Furthermore, Q-PCR showed that the mRNA expression of the histone acetyl transferases HAT1 and MYST3 and the histone methyltransferase EZH2 were upregulated in FASMCF cells.

Conclusions: The study identifies differences in the level of histone marks between Fulvestrant-resistant and responsive breast cancer cells, accompanied by upregulation of genes that have been reported to be responsible for such modifications. Such changes may be contributory to the mechanisms underlying the gene profile of Fulvestrant resistance *in vitro*. The findings suggest combination treatments of Fulvestrant and histone deacetyltransferase inhibitor (HDACI) or demethylating agents, that merit experimental investigation in the context of treating endocrine-resistant breast cancer.

163 Comprehensive Sentinel Lymph Node Examination in Ductal-Carcinoma-In-Situ of the Breast: Much Ado about Nothing
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Background: Sentinel lymph node (SLN) biopsy has gained acceptance as a less morbid alternative in breast cancer management. However, its usefulness in ductal-carcinoma-in-situ (DCIS) is controversial, usually being performed under particular clinical conditions when an invasive component is suspected. In addition, the extent of examination and whether it should routinely include multilevel sectioning and cytokeratin immunostaining (CKI) to detect low-volume metastatic disease is still debated. We present our experience with an extended protocol applied to SLN in patients with mammary DCIS.

Design: A total of 65 DCIS of the breast were accessioned between November 1, 2002, and March 31, 2008. Patients' ages ranged from 43 to 80 years. Average age was 56.9 years; 24 patients (36.7%) were premenopausal. Primary tumors were assessed on core needle biopsy (43 cases) and/or a quadrant biopsy (43 cases). Radical mastectomy was necessary in 28 patients. Formalin-fixed SLNs were usually bisected along the longest axis and 2.0-mm thick slices were submitted in separate tissue cassettes. Each SLN block was step-sectioned at 50-µm (first 15 levels) and then 100-µm intervals (level 16 and over, until block exhaustion), with one section for hematoxylin and eosin (H&E) and one for cytokeratin immunostaining (CKI) using antibody MNF-116. Sections were sequentially numbered in the order they were cut. Immunoperoxidase was ordered in all H&E-negative cases.

Results: In 6 cases (9.2%), microscopic examination of SLNs showed abnormal findings including micrometastases (2 cases) or isolated tumor cells (4 cases). Microscopic findings could be recognized only by means of CKI and were unrecognized on first examination of H&E-stained slides. Axillary dissection was completed in 2 patients with low-volume micrometastases and was negative. No correlation could be observed between SLN results and clinicopathologic parameters, including patient's age, size and extension of DCIS, mammographic features, tumor grade, and estrogen and progesterone receptor status.

Conclusions: Although it may slightly increase the yield of abnormal features, an extended SLN examination protocol is a cost-ineffective and redundant procedure in DCIS, since the gained information is irrelevant to patient care. Whether or not SLN may represent a useful adjunct in selected cases of DCIS, the use of near-total examination methods should be discouraged in routine practice.

164 "Modified" Alcian Blue Enhances the Intraoperative Diagnosis of Sentinel Lymph Node Metastasis in Invasive Lobular Carcinoma: A Prospective Study
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Background: The sensitivity of intraoperative diagnosis of sentinel lymph node (SLN) metastasis of invasive lobular carcinoma (ILC) using conventional staining is low. Multiple studies have used immunohistochemistry and polymerase chain reaction. However, these methods take long time for its purpose and are costly. The goal of our study was to develop a fast "modified" Alcian Blue (MAB) stain to decrease the intraoperative false negativity of metastases of ILC.

Design: A series of Alcian Blue staining modifications was conducted using fresh lobular carcinoma cases. After optimization with the shortest possible period and highest sensitivity, the procedure was used for intra-operative diagnosis (IOD). Patients that had ILC on needle biopsy and scheduled for SLN operation were candidates for the study. Two touch preparations from every SLN were prospectively prepared in the same manner, one stained with Hematoxylin and Eosin (H&E) and one with MAB. These slides were independently interpreted. Finally, all the permanent sections of the lymph nodes were stained with cytokeratin (AE 1/3).

Results: Twenty patients with ILC underwent SLN sampling (Table 1). Approaching data based on number of SLN, there were 16 of 70 (22.9%) positive SLN. The sensitivity for H&E and MAB was 43.8% and 81.3% respectively. Approaching data based on patients, there were 9 of 20 (45%) patients had at least one positive SLN. The sensitivity for H&E and MAB was 55.6% and 88.9% respectively. The specificity for both methods was 100%. The staining process takes 9 minutes.

Conclusions: MAB stain is relatively a rapid, cheap, highly sensitive and specific method of detecting metastatic ILC to SLN. This method can be used in conjunct with normally used intraoperative staining methods.

Results of H&E and MAB based on patients and lymph nodes

	Patient basis			Node basis		
	H & E	MAB	Section IHC	H & E	MAB	Section IHC
Total	20	20	20	70	70	70
Positive results	5	8	9	7	13	16
Negative results	15	12	11	63	57	54
False negatives	4	1		9	3	
False positives	0	0		0	0	
Sensitivity	55.6	88.9		43.8	81.3	
Specificity	100	100		100	100	

165 Validation of the Multiplex Ligand-Dependent Probe Amplification (MLPA) Technique for the Determination of HER2 Gene Amplification in Breast Cancer
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Background: Randomized clinical trials have demonstrated significantly improved outcomes for women with HER2 positive breast cancer treated with traztuzumab in both the adjuvant and metastatic settings. Consequently, it is now mandatory to establish the HER2 status of all breast cancers at diagnosis. The range of testing platforms available includes immunohistochemistry to detect over-expression of the receptor and in situ hybridization (ISH) to establish gene amplification. Given the case volume, PCR based tests are attractive in that they offer the advantages of automation, high throughput and low cost. MLPA is a PCR based assay that quantifies gene copy number, allowing assessment of amplification of the HER2 gene relative to control genes located on chromosome 17 and on other chromosomes. This study aims to compare the results of HER2 gene amplification for breast cancer as assessed by MLPA with CISH and FISH as gold standards.

Design: Institutional ethics board approval was obtained. Using commercially available kits, 208 consecutive invasive breast cancers undergoing routine CISH testing at our laboratory were also tested independently using MLPA. The ACSO/CAP guidelines were applied for the reporting of ISH results. FISH at a central laboratory assessed cases with no signal or equivocal CISH results. Supplementary in-house FISH was carried out as required for the study. In accordance with prior studies, MLPA results were reported as amplified when the HER2 gene copy number per cell was 4.0 or above in any 2 of 3 HER2 probes included in the kit.

Results: At the conclusion of all ISH testing (CISH & FISH) 25 of 208 cases (12.0%) were regarded as amplified, 182 (87.5%) as non-amplified and one case (0.5%) as undetermined due to insufficient tissue. This case is excluded from further analysis. Of the 25 cases amplified by ISH, 23 (92.0%) were classified as amplified by MLPA, the remaining 2 ISH amplified cases were classified as negative by MLPA. Of the 182 cases categorized as not amplified by ISH, all were also classified as negative by MLPA. Using ISH as the gold standard, the sensitivity of MLPA is 92.0%, its specificity 100%, positive predictive value 100%, negative predictive value 98.9% and overall accuracy 99.0%.

Conclusions: The high levels of concordance between MLPA and ISH results merit further consideration of MLPA as a possible additional platform for HER2 testing.

166 Evaluation of RT-PCR for Human Mammaglobin mRNA in Assisting the Diagnosis of Breast Cancer Pleural Effusion
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Background: Detection of breast cancer (BC) cells in pleural effusions (PE) is usually achieved by cytology. However, was reported that the diagnosis of PE by this methodology shows limited sensitivity. Recently, to improve the diagnostic accuracy of BC PE many authors have been evaluated polymerase chain reaction (PCR) and Human mammaglobin (hMAM) was proposed as the suitable mRNA markers. The aim of this study was to investigate the possible application of a nested RT-PCR for hMAM mRNA detection in the diagnosis of BC PE.

Design: Four hundred and thirteen PE samples including 32 from BC, 196 from patients with other cancers and 185 from patients with benign diseases were subjected to nested RT-PCR for hMAM. The results were compared with conventional cytology. Diagnostic performance of hMAM RT-PCR was based on binomial distribution while comparison between correlated proportions was assessed through MCNemar test. Two-tailed p-value<0.05 was considered as statistically significant.

Results: hMAM was found expressed in 114/413 (27.6%) of total PE, in 27/32 (sensitivity of 84.4%) of the PE from metastatic BC, in 77/196 (39.3%) of the PE from other types of cancer (34/77 (44.2%) Lung, 29/80 (36.0%) mesothelioma, 14/39 (35.9%) other cancers) and in 10/185 (5.4%) of the PE from benign diseases. Comparative analysis of RT-PCR and cytology showed that 18 samples from metastatic BC (56.2%) were positive by both PCR and cytology, 9 (28.1%) were positive only by PCR and 5 (15.6%) were negative by both tests whereas no cases were found of positive cytology with negative PCR. RT-PCR increased sensitivity of BC effusion detection of 28.1%

(McNemar test p-value=0.004). The specificity for hMAM detection method was 77.2%, while accuracy, positive predictive value and negative predictive value were 77.7%, 23.7% and 98.3%, respectively.
Conclusions: RT-PCR for hMAM mRNA developed for this study, has provided a rapid, reproducible and cost-effective analysis test for BC diagnosis of PE. The assay was more sensitive but less specific than cytology. We conclude that the test may be a helpful adjunct to cytology for the routine screening of malignant BC effusions.

167 Transcriptomic Profiling of Early Metastatic Transition in Breast Carcinomas
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Background: Lymph node (LN) involvement by tumor cells is the first metastatic event in breast carcinoma and the single most important prognostic factor.Yet, little is known about the molecular events and alterations in biochemical pathways leading to the initial acquisition of the metastatic capacity and the role of hypothetical breast cancer stem cell (CSC) in tumor progression.
Design: Comparative whole-genome transcriptomic profiling of 18 carefully selected pairs of frozen primary invasive ductal carcinoma and their lymph node mestastases as well as 7 non-metastatic tumors was performed on Affymetrix U133 Plus 2.0 chips. Genes differentially expressed among the 3 groups were selected for experimental validation by Reverse Transcription and Quantitative Real-Time PCR (QRT-PCR) with Taqman LDA cards. Laser microdissection was used in a subset of samples in order to confirm the epithelial origin of the transcripts differentially expressed.
Results: Pathway analysis indicated activation of TGF-β and Wnt responses in all primary tumors, but not in metastases. Also, all primary tumors, but not LN metastases, expressed markers and effectors of epithelial-mesenchymal transition (EMT), in particular TWIST1 and SNAI2. In contrast, "stemness" factors such as EZH2, PITX2, SOX12 or SPDEF were more strongly expressed in primary tumors which metastatized, and also in their corresponding metastases, than in primary tumors that had not metastatized. Interestingly, our analysis also showed that all primary tumors (metastatizing and not metastatizing), but not the LN metastases, expressed basal-type keratins 5, 6, 14 and 17, while metastatizing primary tumors and also their LN metastases expressed keratin 18, GATA3 and ESR1, suggestive of a luminal phenotype.
Conclusions: The activation of the TGF-β and Wnt signalling pathways seems to plays a major role in the early acquisition of local invasive phenotypes of breast tumors through induction of EMT, and the capacity of metastasis to the LN is associated with a stronger representation of cells with CSC features.We hypothesize that tumors capable of metastatizing to the LN contain a double tumor cell phenotype (luminal and basal, and also CSC and EMT), which could reflect either the prevalence of a bipotent cell in such tumors, or a mixture of two distinct cell populations.

168 HER2 Over-Expression and Histological Features Correlation in Argentinean Breast Cancer Patients
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Background: HER2 over-expression was found to be an independent overall survival and time to relapse predictor; furthermore, correlates with poor prognostic features variables. HER2 National Program enabled access to HER2 testing country-wide with standardized and replicate technique. Internal as well as independent and external quality control has assured accurate results. **Aim:** To correlate HER2 over-expression in our program population with age and classic histological features, such as: histological type and grade, tumor size and hormone receptors status.
Design: 1702 H&E-stained, formalin fixed paraffin embedded invasive breast carcinoma tissue samples with required criteria were collected. Routine histological parameters were assessed according to WHO Tumor classification. HER2 analysis was performed 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. ER and PR were screened by IHC analyses and interpreted as positive when more than 10% of tumor cells showed positive nuclear staining.
Results: HER2 over-expression in all samples (1702): Score 0-1+:1450 (85,19 %), 2+: 74 (4,34 %), 3+: 178 (10,45 %). **Age and histological features in HER2 - (0-1) versus HER2 + (3) patients: (Table 1)**

Table 1:Age and histological features in HER2 - (0-1) versus HER2 + (3) patients

	HER2-(n1450)	HER2+(n178)	p value(univariate analysis)
Age(median-range)	55 (25-97)	55 (27-88)	0,365
T size cm.(median)	1,8 cm	2,00 cm	0,349
ER+ (%)	1052 (73)	105 (59)	<0,001
PR+ (%)	968 (67)	105 (59)	0,039
GH3 (%)	671 (46)	115(65)	<0,001
GN3 (%)	518 (36)	120 (67)	<0,001
GM3 (%)	133 (9)	29 (16)	<0,001

With univariate analysis: histological, nuclear and high mitotic rate were statistically linked with HER2+ tumors and ER+ were more frequent in HER 2 - tumors. Using logistic regression for multivariate analysis high histological and nuclear grade and less percentage of ER + were independent predictors for HER2 + disease.
Conclusions: HER2 over-expression was positively associated with high histological and nuclear grade and less ER+ in our population in a logistic regression model.

169 Neovascularization in Mucinous Ductal Carcinoma In Situ Suggests an Alternative Pathway for Invasion
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Background: Ductal carcinoma in situ (DCIS) associated with invasive mucinous carcinoma (IMC) has not been well characterized. Our objective is to characterize mucinous DCIS (mDCIS) of the breast and to describe, to our knowledge for the first time, neovascularization in mucin.
Design: We reviewed the pathology reports and slides from 44 patients treated between 2003 and 2006 at The University of Texas M. D. Anderson Cancer Center, whose diagnosis fulfilled the criteria of IMC or DCIS with mucin production.
Results: The patients, all female, had a mean age of 62 years. DCIS was present in 93% of cases, and the predominant histological types were solid , cribriform , and micropapillary. The DCIS was nuclear grade 1 in 12 of 41 cases (29.3%), grade 2 in 25 of 41 cases (61%), grade 3 in 4 of 41 cases (9.8%). Mucin was seen in the lumen of the ducts involved by DCIS in 88% of cases, mucin and vessels in 63.4% of cases and neither mucin nor vessels in 12.2%. The DCIS was VEGF and PDGFRbeta positive and CDX-2 negative (100%). CD68 stained occasional luminal cells within the DCIS.
Conclusions: A significant number of mDCIS showed neovascularization in intraluminal mucin. When identified on core needle biopsy, the presence of vascularized mucin should not be used alone to discriminate between invasive and in situ carcinoma. A hypothesis proposed for the source of recruitment of vessels in the mucin is that mucin can promote neovascularization and that tumor cells invade not into the adjacent fibroconnective tissue , but rather into the mucinous richly vascularized stroma that they have induced. Alternatively, it is possible that both cells and their secretory product invade together. To our knowledge, this is the first study to characterize neovascularization within the mucinous component of DCIS associated with and without IMC.

170 D2-40 Reactivity in Spindle Cell Lesions of the Breast
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Background: The D2-40 antibody to podoplanin is a marker first described in lymphatic endothelium which has been noted in many malignant tumors including angiosarcomas and mesotheliomas, as well as normal tissues, including stromal cells of the breast. Given the potential considerations among spindle cell lesions of the breast and chest wall, characterization of D2-40 expression has diagnostic relevance.
Design: Our electronic database was searched for all primary breast lesions seen over a 12 year period that were described as metaplastic, spindle cell, sarcomatoid, or sarcomatous. Seven representative examples of low-grade phyllodes tumors were also included. Immunohistochemistry for D2-40 was performed and blindly scored for quantity (0, none; 1,<10%; 2,10-50%; 3, >50%) and quality (0,negative; 1, mild at 20x; 2, strong at 10x; 3, very strong at 2x). These values were multiplied and a resulting score of at least 6 was considered positive. Clinicopathologic data were collected on each case and reviewed, including IHC performed during routine clinical evaluation.
Results: 3 of the 7 metaplastic carcinomas were immunoreactive with D2-40. One of the positive cases plus one of the negative cases demonstrated partial reactivity for other vascular markers, and had been considered to represent pseudovascular carcinomas. Only 1 of 5 low-grade sarcomas, and none of the low-grade phyllodes tumors (n=7), or high-grade sarcomas without a history of local radiation (n=5) was positive. None of these was vasoformative or had documented reactivity for another vascular marker. All 5 high-grade sarcomas associated with past radiation for breast carcinoma were positive. Three of these had vasoformative areas plus reactivity for CD31. These features were lacking in the other two tumors, both of which demonstrated strong SMA staining.
Conclusions: Although spindle cell lesions of the breast are generally negative for D2-40, a subset of metaplastic carcinomas and radiation-induced sarcomas are positive, with or without supportive evidence of vascular differentiation.

171 Significance of Lobular Carcinoma In Situ (LCIS), "Nuclear Grade 2" on Breast Core Needle Biopsies
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Background: LCIS is regarded as a risk factor for development of subsequent invasive carcinoma (IC). It's still unclear at this time, the role of LCIS as a precursor lesion. Though the literature on LCIS is emerging, there are many questions still unanswered. The current treatment of LCIS includes a close follow-up and consideration for tamoxifen treatment for reduction of cancer risk long term in these patients. Many variants of LCIS have been described based on pathologic features such as nuclear grade, pleomorphism and necrosis but very little is known about the biology of these variants. The proposed three tier grading system classifying LCIS has not been validated nor endorsed across the laboratories. We found a significant upstaging of pure pleomorphic LCIS (LCIS with nuclear grade 3) upto 25% in CNB (*chivukula et al*). The aim of our study was to address the importance of this ambiguous nuclear grade 2 (LCIS-NG 2) diagnosed on CNB along with clinicopathologic follow-up.
Design: The co-path pathology report archives using natural language search in the Department of Pathology were searched for the period of 2006-2008 to include the surgical pathology reports for consecutive breast core biopsies during a 2-year period. Cases in which pure LCIS, nuclear grade 2 in the diagnosis were selected and reviewed. All cases with associated ductal carcinoma in situ (DCIS) or IC (ductal or lobular) were excluded. The clinical, pathologic and radiologic follow-up data was obtained.
Results: Total number of CNB performed from 2006-2008 was 8054. Based on the strict criteria, 27 cases of pure LCIS, nuclear grade 2 were selected. Radiologic biopsies performed were in majority stereo-tactic guided (78% (21/27), remainder were MRI and ultrasound guided. Though all stereotactic biopsies were performed for calcifications (ca++), 93% (25/27) showed presence of Ca++. In the follow-up resections, DCIS or IC was seen in 11% (3/27), residual LCIS seen in 48% (13/27) cases and other high

risk lesions such as atypical ductal hyperplasia were seen in 37% (10/27) of cases. No follow-up was available in 19% (5/27) cases.

Conclusions: 1. A great majority of these cases w/ LCIS –NG 2 are associated with microcalcifications. **2.** The upstaging to a more significant lesion in comparison to pleomorphic LCIS is lower (48% versus 11%), **3.** Follow-up excision is recommended in patients with LCIS-NG 2 due to residual LCIS, and associated high risk lesions resulting in upstaging.

172   Ductal Carcinoma In Situ in Women under Age of 35
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Background: DCIS is rare under age of 35 years. The evolution of invasive carcinoma (IC) in this age group may be different from that in older women and is not well understood. The high frequency of triple negative/basal vs. luminal phenotype in young may indicate differences or even absence of traditional precursor lesions in this population. We retrospectively reviewed the clinical, morphologic and phenotypic characteristics of DCIS in women <35 years old.

Design: We identified 66 women <35 years presenting with breast carcinoma diagnosis from 2005- 2007. All cases with DCIS (with or without invasive component) were included. We reviewed the morphology, existing clinical data, and the immunohistochemistry (IHC) profile including ER, PR, HER-2, p53, Ki67, and CK5/6.

Results: Study cases included 19 invasive carcinoma (IC), 38 DCIS with IC (DCIS+I), and 9 with pure DCIS. Pure DCIS represented 13% of carcinomas in <35 age group. Patients age ranged 22-35 years (mean=32 years). 89% of DCIS patients were asymptomatic while 78% of DCIS+I presented with nipple discharge or mass. Overall, 25% of all DCIS patients had family history of breast carcinoma with high frequency of BRCA (4 positive/8 tested). History of pregnancy or lactation at diagnosis was only present in DCIS-I group (21%). Mean size of in situ tumor was 1.6 cm (0.3-6cm) for DCIS and 3.5 cm (1.0-8.4 cm) for DCIS+I. DCIS+I were extensive (represented >25% of tumor volume) in 83% (30/36). Nuclear grade of DCIS was 3 in 34/47 (72%). Compared to a group of 278 consecutive DCIS cases diagnosed in 6 months period high nuclear grade was significantly more common in DCIS+I group (Chi square, p<0.01), but not in DCIS group

DCIS vs. DCIS-I vs invasive carcinoma: immunohistochemical phenotype and DCIS grade

	Luminal A,B	HER-2+	Triple -	KI-67>10%	DCIS grade 1/2/3
Invasive (n=19)	53%	18%	29%	N/A	N/A
DCIS+I (n=38)	65%	16%	19%	80%	3/18/79 (%)
DCIS (n=9)	86%	14%	0	17%	10/45/45 (%)

Basal-like phenotype (triple-/CK5/6+) was seen in DCIS+I (3/38, 8%) but not in DCIS.

Conclusions: The results suggest the possibility that two types of DCIS exist in young women; one with high nuclear grade, high proliferation index, and phenotypes similar to invasive carcinoma that quickly progresses to invasion. The second type of DCIS presents as an asymptomatic lesion, not associated with pregnancy or lactation, is ER+ with low proliferation rate and needs longer time to progress to invasive carcinoma.

173   Assessments of *HER2* Gene Status in Breast Carcinomas with Polysomy of Chromosome 17
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Background: Fluorescent in-situ hybridization (FISH) analysis is used to assess *HER2* gene copy number and detect gene amplification as a part of clinical algorithm for treatment of breast cancer patients. Given the significant clinical benefits of trastuzumab therapy available to patients with HER2-positive breast cancer, it is of paramount importance to accurately identify all patients eligible for this therapy. The *HER2* gene is located on chromosome 17q11.2-q12. Interpretation of FISH assays depends on scoring the precise number of HER2 hybridization signals per tumor cell nucleus. Current guidelines define HER2-positive tumors by FISH as having an average *HER2* gene copy number of more than 6 copies per nucleus or as a *HER2* gene to chromosome 17 ratio of more than 2.2.

Design: Commercially available Locus Specific Identifier (LSI) *HER2* DNA probe (190 Kb Spectrum Orange directly labeled fluorescent DNA probe) and the Chromosome Enumeration Probe (*CEP*) 17 DNA probe (5.4 Kb Spectrum Green directly labeled fluorescent DNA) were used for FISH (PathVysion, Abbot Molecular) on formalin fixed paraffin embedded breast cancer cases. Polysomy 17 was defined as three or more *CEP17* signals per nucleus (average for 30 cells). HER2 protein expression was investigated using standard immunohistochemical methods.

Results: Polysomy 17 was detected in 73 cases (12% of all tested patients). Average *CEP17* copy number was 4.54 (range 3.0 to 10.4). 19 cases (26%) qualified as unequivocally *HER2* gene amplified using *HER2/CEP17* ratio (>2.2) guidelines, and 33 cases (45%) had on average 6 or more *HER2* gene signals per nucleus. Ten cases with 6 or more *CEP17* had concomitant *HER2* gene amplification (HER2/CEP17 > 2.2) in 4 cases. Chromosome 17 polysomy showed no correlation with HER2 protein expression, but a positive trend was observed between HER protein expression and *HER2* gene amplification (*HER2/CEP17* ratio). A positive association between polysomy 17 and higher cancer stage was observed.

Conclusions: A substantial proportion of breast carcinomas harbor multiple copies of chromosome 17. Potentially clinically significant polysomy 17 (>6) is seen in 45% of cases, which could result in 42% discordant interpretation between absolute *HER2* copy number and *HER2/CEP17* gene amplification ratio. A minor fraction of carcinomas with polysomy 17 overexpresses HER2 protein, usually associated with *HER2* gene amplification (*HER2/CEP17* ratio>2.2), indicating that ratio is probably predictive of a therapeutic response in this subgroup of patients.

174   Differential Copy Number Aberrations in New Candidate Genes Associated with Early Breast Cancer Progression
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Background: We recently reported our results of a study identifying a number of DNA copy number changes that differentiated low grade ductal carcinoma in situ (DCIS) lesions with and without associated stromal invasion. We now describe a series of validation experiments that support the role of eight genes in early breast cancer progression, most of which were not previously implicated in mammary neoplasia.

Design: DNA was extracted from microdissected paraffin sections of formalin fixed low grade DCIS lesions with an invasive component (n=25), as well as morphologically similar DCIS lesions without associated invasion within at least 5 years (n=20). The DNA was used for quantitative PCR analysis. Specific primers were designed for nineteen candidate genes. Additional validation studies employed genomic DNA samples from an independent cohort of 35 microdissected DCIS lesions with (n=17) and without (n=18) an associated invasive component.

Results: Our previous profiling study revealed that DNA copy number changes at several chromosomal loci may occur at different rates in low grade DCIS lesions with and without associated stromal invasion. From the differentially amplified or deleted chromosomal sites, we selected nineteen candidate genes for validation by comparative PCR. Eight of these genes were confirmed to have differential copy number changes. GRAP2 (on 22q) was more often amplified in DCIS with associated invasion, whereas TAF1C (on 16q) was more commonly deleted in pure DCIS lesions. NCOR2/SMRT and NR4A1 (both on 12q), DYNLRB2 (on 16q), CELSR1, UPK3A and ST13 (all on 22q) were more frequently amplified in pure DCIS lesions. Moreover, NCOR2/SMRT, NR4A1 and DYNLRB2 showed more frequent copy number losses in DCIS lesions that had progressed to invasive carcinoma. For NCOR2/SMRT, these observations were confirmed in an independent cohort of 35 microdissected low grade DCIS cases. The other eleven candidate genes displayed significant frequencies of copy number changes that were not differentially distributed, however.

Conclusions: Our validation experiments have provided evidence for the role of novel genes in early breast cancer progression. Six of the eight candidate genes may function as invasion suppressors. Loss of NCOR2/SMRT was associated with stromal invasion. This gene was previously implicated in metastasis.

175   Microarray CGH Analysis Reveals Genetic Heterogeneity between Distinct Components of Metaplastic Breast Carcinomas
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Background: It has been suggested that breast cancer may be composed of multiple populations of sub-modal clones harbouring the same initiating genetic lesions followed by the acquisition of additional divergent genetic hits. We analysed the genome-wide genetic features of distinct components of metaplastic breast carcinomas (MBCs) to determine whether these components would harbour divergent genetic aberrations.

Design: The distinct components of three carcinomas with heterologous elements (chondroid, spindle and/or epithelial), one biphasic spindle cell carcinoma (spindle and ductal) and one adenosquamous carcinoma (squamous and lobular) were microdissected. DNA was extracted and subjected to microarray comparative genomic hybridisation analysis using a 32K tiling path bacterial artificial chromosome array platform, which has a resolution of 50kb. Representative sections of each case were subjected to immunohistochemistry with antibodies against oestrogen (ER) and progesterone (PR) receptors, HER2, epidermal growth factor receptor (EGFR), cytokeratins (Cks) 5/6 and 14.

Results: The three carcinomas with heterologous elements lacked ER, PR and HER2 expression and expressed Ck 5/6 and/ or Ck14 and/ or EGFR. At genetic level, the components of these lesions displayed identical genetic profiles. The biphasic spindle cell carcinoma lacked ER and PR, and expressed HER2 2+ and basal markers. The components of this case harboured similar gains and losses and amplification of 9p23 and 17q11.2 (*HER2*); however, only the spindle cell component displayed amplification of 3q24 (*ZIC1*), 5p15 (*TGAT*), 9p24 (*GLIS3*), 10q21 (*CDK1, EGR2*) and 15q23 (*PKM2*), whereas only the epithelial component harboured amplification of 8p11.2 (*MYST3*), 12q14 (*CDK4, SAS* and *GLI1*), 15q26 and 17q23 (*CAS3, TBX2, TBX4*). In the adenosquamous carcinoma the lobular component was positive for ER and PR, and negative for HER2 and basal markers; whereas the squamous component lacked ER, PR and HER2 and expressed basal Cks. Only the squamous component harboured amplification of 7p11.2 (*EGFR*) and displayed overexpression of EGFR 3+.

Conclusions: Our results provide evidence that in some MBCs, morphological intra-tumour heterogeneity can be ascribed to divergent genetic aberrations, with specific high level amplifications of regions encompassing oncogenes known to play a role in breast cancer.

176   Increased Lymphatic Vessel Density in Positive Sentinel Lymph Nodes Predicts Additional Nodal Disease in Breast Cancer
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Background: The accuracy of sentinel lymph node (SLN) biopsy for predicting the status of axillary lymph nodes in clinically node negative breast cancer patients has been confirmed. While patients with negative SLN need no further axillary surgery, for patients who have positive SLNs, completion axillary dissection (CALND) remains the standard practice. Although the SLNs are the only positive nodes in 50-65% of these patients, currently no reliable means exist to identify patients who could be spared the potential morbidity of CALND. We investigated whether increased lymphatic vessel density (LVD) in positive SLNs can help predict the presence of additional nodal disease.

Design: We selected 127 breast cancer cases with positive SLN biopsy for the study. Lymphatic vessels in positive SLNs were detected by D2-40 immunohistochemistry and the LVD at the periphery of metastatic tumor clusters was determined by the hot-spot method. LVD in the SLNs was correlated with clinicopathologic tumor features and the presence of additional nodal metastases in subsequent CALND.

Results: SLN metastasis was confined to 1 SLN in 75 (59%) and involved more than 1 SLN in 52 (41%) cases. One hundred cases (79%) showed macrometastases, while only micrometastases were seen in 27 (21%) cases. LVD in the positive SLNs showed no correlation with tumor type, histologic grade, primary tumor size, or hormone receptor and HER2/neu expression. A significant positive correlation was seen between LVD and the size of metastatic disease in SLNs; LVD was significantly higher in SLNs involved by macro- compared to micrometastases (p<0.0001). Additional nodal disease in subsequent CALND was present in 61 (48%) cases. LVD in positive SLNs was significantly increased in cases associated with additional nodal disease on subsequent CALND both among all cases (p<0.0001) and when cases with only single positive SLNs were analyzed separately (p=0.0008). Using a cutoff at the median LVD value, the sensitivity and specificty of high LVD to predict additional nodal disease were 0.7143 and 0.7344, respectively.

Conclusions: Our results suggest that increased lymphangiogenesis as measured by LVD in SLNs involved by metastatic disease may play a significant role in further lymphatic spread of breast carcinoma. Assessment of LVD in positive SLNs may help in selecting patients who can be spared CALND and its associated potential morbidity.

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177 Immunoexpression of Fli-1 by Metaplastic Breast Cancer: A Potential Pitfall in Cases Morphologically Resembling Epithelioid Angiosarcoma
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Background: Metaplastic breast carcinoma may exhibit spindled or vasoformative morphology that resembles epithelioid angiosarcoma. This may cause particular diagnostic difficulty in core biopsies of the breast. Immunoexpression of epithelial markers can be demonstrated in many metaplastic breast cancers but also in a minority of epithelioid angiosarcoma. Immunoexpression of vascular markers (Fli-1, CD31, CD34) characterize angiosarcoma, but Fli-1 immunoexpression in metaplastic breast cancer has not been formally defined. In this study, we compare the diagnostic utility of Fli-1 immunoexpression to distinguish metaplastic breast cancer with angiosarcomatous features from epithelioid angiosarcoma.

Design: Formalin-fixed, paraffin-embedded tissue sections were selected from 14 metaplastic breast cancers that resembled angiosarcoma. Eleven cases of epithelioid angiosarcoma (from either soft tissue or breast origin) were selected for comparison. Immunostaining was performed for Fli-1 (BD Biosciences, 1:50), CD31 (Biogenex, 1:4), CD34 (Novocastra, 1:400), high molecular weight keratin (HMWK 903, Enzo, 1:2), Keratin MNF116 (DAKO, undiluted), and p63 (Labvision, 1:400). Nuclear expression of Fli-1 and p63 was defined as positive. Cytoplasmic or membrane expression of the remaining markers was defined as positive.

Results: The proportion of metaplastic breast cancers expressing Fli-1 (9/14, 64%) was not significantly different than Fli-1 expression by epithelioid angiosarcoma (10/11, 91%) (p = 0.16), however, the intensity and distribution of expression was markedly different. In metaplastic breast cancer, Fli-1 was weak and patchy in most cases whereas in all epithelioid angiosarcoma, Fli-1 was diffuse and intense. Keratins 903 and MNF-116 were expressed in 14/15 and 13/13 metaplastic breast cancers and in 3/11 and 3/11 epithelioid angiosarcomas, respectively. p63 was expressed in 6/9 metaplastic breast cancers and 2/11 epithelioid angiosarcomas. None of the metaplastic breast cancers expressed CD34 but in 2 cases CD31 was expressed in scattered rare tumor cells.

Conclusions: Although Fli-1 immunoexpression can be detected in metaplastic breast cancer, the staining intensity and distribution is minimal and should not be interpreted as evidence of epithelioid angiosarcoma. A broad panel of epithelial markers, p63, and other vascular markers should be used instead of relying on Fli-1 alone to diagnose angiosarcoma in the breast.

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178 Hormone Receptor Immunohistochemistry: The Prevalence of ER-PR+ Results Are PR Antibody Clone-Dependent
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Background: One of the single-positive categories of hormone receptors (HR) by IHC is the ER-/PR+ group. This category was documented with the ligand-binding method of HR assay and comprises a small fraction cases by immunohistochemistry (IHC). Some argue that poor tissue fixation yielding a false-negative ER is the reason for these results. We have previously demonstrated that fixation is not related to the negative ER result (Mod Pathol. 2008;21(supp 1):27A-abstract 111). To further characterize the nature of the ER-/PR+ category, we compared two ER clones and three PR clones to ascertain whether the prevalence of the ER-/PR+ category is clone driven.

Design: One hundred and ten ER-/PR+ cases from a 30 month period, Jan 1, 2005-June 30, 2007 were available for study, comprising 5.7% of the total cases for this time period. Criterion for a positive result for ER or PR was any nuclear expression. These results utilized ER 6F11 and PR 1A6 clones on the Benchmark XT according to FDA kit protocols. The same cases were studied with ER clone SP1 and PR clone 1E2 on the Benchmark XT with FDA kit protocols. In addition PR clone 636 on the Dako autostainer with FDA protocol was also performed. Results were tabulated according to "any nuclear expression" and >1% of cells with nuclear expression.

Results:

	Any + Nuclei (No. of cases/%)	>1% + Nuclei (No. of cases/%)
PR 1A6	110/100	39/35
ER 6F11	0/0	0/0
ER SP1	13/12	9/8
PR 1E2	19/17	9/8
PR 636	28/25	6/5

Conclusions: (1) ER-/PR+ cases are seen by the IHC method even with optimal fixation methods. (2) The number of ER-/PR+ cases is highly dependent upon the clone of PR used, with clone 1A6 yielding the highest percentage, more than four-fold compared to the 1E2 and 636 PR clones. (3) The method of determining a positive result -any cells positive vs >1% of cells positive also has an impact on ER-PR+ metrics. (4) SP1 ER clone yields 8% more positive cases compared to ER 6F11, a result comparable to data previously published (Cheang MC et al J Clin Oncol 2006;36:5637-44).

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179 Detailed FISH Analysis of HER2 2+ Immunohistochemistry Cases: Rarity of Cases with High Level HER2 Gene Amplification
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Background: One-quarter of 2+ immunohistochemistry (IHC) cases are amplified by fluorescence in-situ hybridization (FISH), but amplification details (low-level versus high level gene amplification, aneuploidy/hyperploidy, monosomy) for these cases remains unknown. These details may be important for gauging individual patient response to targeted therapy.

Design: The HER2 IHC was performed either using Pathway (clone CB11) HER2 antibody or 4B5 clone. One hundred and eighty-six consecutive invasive breast carcinomas 2+ by IHC were analyzed by FISH for *HER2* gene amplification using PathVysion dual color probe (Vysis Inc., Downers Grove, IL). FISH results were categorized into 3 categories (Amplified, not amplified and equivocal) using CAP-ASCO guidelines. Cases with average Chromosome enumeration probe 17 (CEP17) signals of >3 were considered as positive for aneuploidy/polyploidy. Monosomy for chromosome 17 was suspected when majority of the cells showed only one signal for CEP17.

Results: Of the 186 cases, 150 (81%) were not amplified, 21 (11%) were amplified, and 15 (8%) were equivocal for *HER2* gene amplification. Aneuploidy/polyploidy was identified in 20 of 150 (13%) non-amplified cases. Among the 21 amplified cases, the mean *HER2*/CEP17 ratio was 3.08 with mean *HER2* gene copy number/cell of 6.4. Only 2 of 21 amplified cases (10%) demonstrated clusters of *HER2* gene. Five of 21 amplified cases (24%) showed heterogeneity with respect to *HER2* gene copy number/cell. Three of 21 amplified cases (14%) showed monosomy which was responsible for *HER2*/CEP17 ratio of >2.2. If a single colored probe was used, then 3/21 (14%) amplified cases would have been classified as not amplified and 7 (33%) cases as equivocal. Of the 15 equivocal cases, 4 cases (27%) showed monosomy. If a single colored probe was used, then 6 (40%) of the original equivocal cases would have been classified as not amplified.

Conclusions: 1) The number of 2+ IHC cases with *HER2* gene amplification is slightly lower than previously reported with the use of an equivocal category. 2) Large clusters of *HER2* gene/cell (a characteristic of 3+ IHC cases) are very uncommonly seen in IHC 2+ cases. 3) Aneuploidy/polyploidy for chromosome 17 without *HER2* gene amplification may explain 2+ IHC staining in 13% cases. 4) Depending on the type of probe (single versus dual color), the interpretation may vary-Dual color probe is useful in detecting ploidy, however has the potential for artifactually elevating the *HER2*/CEP17 ratio, which should be interpreted appropriately.

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180 Basal-Like Breast Carcinoma: Identifying Estrogen Receptor Positive Cases
DC Gingell, S O'Connor, R Bhargava, DJ Dabbs. Magee-Womens Hospital of UPMC, Pittsburgh, PA.

Background: Basal-like (BL) breast cancer is one of the most aggressive in terms of prognosis. BL breast cancer has been characterized on the basis of gene expression, but it is becoming common for these tumors to be defined on the basis of immunohistochemical (IHC). The "triple negative" (TN) phenotype lacks immunostaining for ER, PR, and HER2, and is considered a surrogate marker for BL breast cancer. Results of these immunostains are unique in driving therapeutic decisions. Sensitivity of ER antibody clones varies, and low levels of ER expression may not be detected by all clones. Given the therapeutic implications, it is important to determine if any ER clones are more sensitive for the detection of ER.

Design: The study was performed on 27 BL breast carcinoma tissue microarrays (TMAs). Each tumor was represented by 3 cores, with each core measuring 0.6mm in diameter. The BL carcinomas represented on TMA have been previously identified as triple negative tumors using 6F11 ER clone. These tumors have further been characterized by being positive for at least two of the following BL tumor markers: CK5/6, CK14, CK17 and EGFR. The tumor TMA were stained with ER clones 6F11 (Ventana Medical Systems), 1D5 (DAKO), and SP1 (Ventana Medical Systems). Whole tissue sections for 12 of the 27 TMA cases were stained using all three ER clones. H-score methodology was used to calculate scores for IHC stains. This method takes into account percentage as well as intensity of staining. The score ranges from 0-300. Any staining was considered positive.

Results: All 27 TMA cases were negative for 6F11 ER clone, where as 9 (33%) of these cases showed some reactivity using either 1D5 or SP1 clones. The average H-score for 1D5 cases was 5.2 (range 1-10); the average H-score for SP1 cases was 5.4 (range 2-10). The following table reflects the concordance between the clones:

	1D5+	1D5-
SP1+	2	3
SP1-	4	18

Whole tissue sections (WTS) of 12 of the 27 TMA cases showed similar ER staining as the corresponding TMA in 6 of the 12 cases. Of the discordant cases, 4 were due to some positivity seen in the WTS whereas the TMA was negative.

Conclusions: 1) Some BL breast carcinomas, identified using IHC markers, show weak ER positivity. Using the threshold for positivity as any cells positive, these cases may not be identified as BL. 2) The number of ER+ BL cases identified is dependent upon the clone of ER used, with 1/3 of the 6F11 ER negative cases being ER+ using either 1D5 or SP1. 3) The choice of clone may fail to identify some ER+ patients who would benefit from adjuvant tamoxifen.

181 Reliability of Chromogenic In Situ Hybridization (CISH) for Detecting HER2 Gene Status in Breast Cancer: A Study of Two Institutions Using Manufacturers' Scoring Criteria and the New ASCO/CAP Recommendations
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Background: The new ASCO/CAP guidelines mandated that a new HER2 assay should show ≥95% concordance with another validated test for positive and negative results before routine application. Chromogenic in situ hybridization (CISH) has recently showed a potential to replace fluorescence in situ hybridization (FISH) to determine the HER2 gene status, owing to being relatively straightforward and simpler on interpretation. However, the reliability of CISH method using the new ASCO/CAP scoring criteria has rarely been addressed.
Design: Paraffin tissues of 226 consecutive cases of surgically resected invasive breast carcinomas were retrospectively obtained from two institutions: 110 from M. D. Anderson Cancer Center (site A) and 116 from University of Tampere (site B). We tested CISH and corresponding FISH on the same set of 226 tumors simultaneously at both sites. The results of each test were interpreted by pathologists at each site. Both the new ASCO/CAP guidelines and manufacturers' scoring criteria were used to interpret CISH and FISH results.
Results: Using the manufacturers' scoring criteria, the concordance between CISH and FISH was 98.5% at site A and 98.6% at site B, and the reproducibility of CISH results between the two sites was 99.0%. When a three-category criterion (amplified, equivocal and non-amplified) defined by ASCO/CAP guidelines was used, the concordance between the two methods was 95.0% at site A and 96.4% at site B, and the reproducibility of CISH results between sites was 98.5%. When the two-category criterion (ie, excluding equivocal cases) was used for comparison, the concordance between the two methods was 99.0% at site A and 99.1% at site B, and the intersite agreement on CISH results was 100%.
Conclusions: High correlation was oberved between CISH and FISH at the two sites, using manufacturers' and the ASCO/CAP scoring criteria. The concordance exceeds the minimum concordance rate (95%) mandated by the ASCO/CAP for positive and negative results. This study also showed excellent reproducibility of CISH results between test sites.

182 Validation of Dako DuoCISH™ *HER2* Gene Amplification Assay
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Background: Over-expression and amplification of *HER2* have been identified as poor prognostic factors in breast cancer and as predictive markers of response to targeted therapy. Standard laboratory methods used to assess *HER2* status include immunohistochemistry (IHC), fluorescent in situ hybridization (FISH) and chromogenic in situ hybridization (CISH). Until now, the only commercially available kit for CISH used single color visualization of a labeled *HER2* probe and lacked a probe for chromosome 17. Inclusion of a chromosome 17 probe is important to distinguish pseudo-amplification of the *HER2* gene (polysomy of chromosome 17) from true gene amplification. The aim of this study was to validate a new commercially available CISH *HER2* gene amplification assay (DuoCISH™, Dako) that allows for dual color chromogenic visualization of signals from both *HER2* and chromosome 17 probes.
Design: Breast cancer and normal tissue microarrays (TMAs) containing cores from 131 patients at one University Hospital were constructed. *HER2* status was determined by validated IHC (Herceptest™, Dako) and FISH (PathVysion® Vysis), and compared with CISH (DuoCISH™, Dako). All assays were performed and interpreted (blinded to other assays) according to the manufacturers guidelines. The results of CISH were compared to both IHC and FISH.
Results: A comparison of CISH and IHC (71) showed 100% agreement between CISH amplified and IHC 2⁺-3⁺, n=7; and 100% agreement between CISH not amplified and IHC 0-1⁺ (negative), n=59. Some cores read as CISH equivocal were IHC 0-1⁺, n=7. Comparing CISH and FISH (63) demonstrated 100% agreement between CISH not amplified and FISH ratio <1.8 (not amplified), n=51; and 71% agreement between CISH amplified and FISH ratio >2.2 (amplified), n=5/7. 5 cores read as CISH equivocal were FISH ratio <1.8 (not amplified). Comparing IHC and FISH (83) there was 100% agreement between IHC 0-1+ (negative) and FISH ratio < 1.8 (negative), n=76; and 100% agreement between IHC 3+ (positive) and FISH ratio >2.2 (amplified), n=4. Of the 3 cores read as IHC 2+, 2 were FISH ratio <1.8 (negative) and 1 was FISH ratio >2.2 (amplified).
Conclusions: The DuoCISH *HER2* gene amplification assay (Dako) showed high concordance with IHC and FISH and should be considered for use in the assessment of *HER2* status in breast cancer. CISH equivocal cases may need further evaluation.

183 Achieving 95% IHC/FISH Concordance for Her2: Causes and Implications of Discordant Cases
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Background: Her2 oncogene overexpression predicts treatment response to multiple therapies, most notably trastuzumab. ASCO-CAP consensus recommendations published in 2007 require Her2 IHC concordance with either a reference IHC sample or another validated Her2 assay, such as fluorescent in-situ hybridization (FISH). This benchmark dictated a higher concordance rate than many published articles report. While compiling concordance data at the University Of Washington Medical Center (UWMC), our concordance studies between IHC and FISH for 2007 showed >99% concordance on IHC 0/1+ cases and 96.5% concordance on IHC 3+ cases. We expanded our concordance studies to include cases from 3 consecutive years and investigated reasons for discordant cases.
Design: All cases having both IHC and FISH performed between 2005 and 2007 were analyzed. Discordant reported results were reviewed and patient histories were obtained when possible. IHC was performed using anti-Her2 antibody A0485 (Dako) and a "home-brew" procedure, with interpretation relying on 'subtraction' of the staining of non-neoplastic ducts from neoplastic ducts. FISH was performed using a validated commercial assay (Abbott-Vysis). When cancers were composed of subpopulations having different IHC and/or FISH expression, each subpopulation was treated as a separate case.
Results: From 2005-2007, 703 cases had both IHC and FISH studies performed. 14% were IHC positive, 31% were equivocal, and 54% were IHC negative. Overall concordance of IHC 0/1+ with FISH (-) was 98% (n= 364 cases) and with IHC 3+ and FISH (+) was 84% (n= 96 cases). Discordant cases included 15 that were IHC 3+, FISH (-). Of these, 9 would not have been interpreted as 3+ using the 2007 CAP/ASCO criteria; 2 others lacked internal controls. Hence, 2007 criteria improved concordance for IHC 3+ from 84% to 95%. Six other discordant cases were IHC 0/1+, FISH amplified (concordance for IHC 0/1+ is 99%). At least 2 of these patients were treated with trastuzumab before biopsy.
Conclusions: Overall concordance between IHC and FISH is high and ASCO/CAP criteria for IHC interpretation significantly improve concordance for IHC 3+ carcinomas. Even with revised criteria, discordant cases remain. Concordance is excellent for IHC 0/1+ FISH (-) cases and notably, some discordant cases in the latter group may relate to prior trastuzumab therapy. We propose that such cases be excluded from concordance studies.

184 Can ER and CK5 Identify Atypical Epithelial Proliferations in Papillary Lesions of the Breast on Core Biopsy?
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Background: The presence of atypical or usual epithelial proliferations within papillary breast lesions complicates their interpretation on core biopsy. Although histologic features are paramount, we evaluated the combination of estrogen receptor (ER) and cytokeratin 5 (CK5) as an aid in the distinction of usual duct hyperplasia (UDH) from atypical proliferations in this setting.
Design: Core biopsies from 185 papillary lesions were reviewed and of these,82 cases were selected for immunohistochemical study based on the presence of an epithelial proliferation between the fibrovascular cores. Fifty-two cases were used as the test set and 30 cases, with subsequent surgical excision, were used as the validation set. The epithelial proliferation was evaluated for staining intensity and percentage of positive cells using CK5 and ER.
Results: Of the test set, a consensus diagnosis of benign papilloma with UDH was reached in 20 cases and atypical papillary lesion (either limited (ADH) or more extensive (DCIS)) in 32 cases. Expression of both CK5 and ER was significantly different in benign lesions when compared with atypical lesions (p<0.0001). Benign lesions typically showed an ER-low/CK5-high profile and atypical lesions showed an ER-high/CK5-low profile with ER-high expression defined as diffuse strong staining in >90 % of cells. Anything less or variation in intensity was considered ER-low. CK5-high expression was defined as a mosaic pattern of staining in >20% of cells and CK5-low as absent or staining in < 20% of cells. Based on their staining profile 29 of the 30 validation cases were correctly classified using the excision specimen as the gold standard (Table 1). The remaining case did not fit either the benign or atypical staining profile.

Table 1.Staining profile of validation set according to diagnosis on excision specimen.

Excision diagnosis	ER-high/CK5-low N (%)	ER-low/CK5-high N (%)	ER-low/CK5-low N (%)	ER-high/CK5-high N (%)
Benign	0	15/15 (100%)	0	0
Atypical/DCIS/Invasive	14/15 (93%)	0	1/15 (7%)	0

Atypical profile = 93% sensitive, 100% specific;Benign profile = 100% sensitive, 100% specific.
Conclusions: Patterns and extent of ER and CK5 staining, when used together, are valuable adjunct stains to differentiate UDH from atypical proliferations within papillary lesions on core biopsy. This is the first report to illustrate the value of the combined use of ER and CK5 in the characterization of epithelial proliferations of breast.

185 Molecular Analysis of Invasive Micropapillary Carcinoma of the Breast
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Background: Invasive micropapillary carcinoma (IMPC) of the breast is a rare entity, associated with frequent axillary lymph node metastasis and vascular invasion and has recently been identified as being luminal.

Design: To get insight the molecular alterations of IMPC, we performed array CGH and transcriptomic (Affymetrix U133 2+) analyses of 25 IMPC and compared them with 25 cases of estrogens positive invasive ductal carcinomas (IDC), all with vascular (LVI) and axillary lymph node invasion (N+). An immunohistochemistry analysis was performed with antibodies against estrogens (ER) and progesterone (PR), ERBB2, MUC1 and E-cadherin.
Results: The majority of IMPC was N+ (69%), LVI+ (84%), grade 2 (64%), ER positive (92%), and were ERBB2 positive in 6/25 cases (24%). All cases demonstrated an inverted apical pole MUC1 positive/E-cadherin negative. IDC were all N+, LVI + and ER +, in majority grade 2 (15/25 cases 60%) and 2 cases were ERBB2 3+ (8%). Genomic analyses showed that both groups shared gains of 1q, 8q24, 16p and 20q and losses of 16q (observed in up to 18/25 (72%) of IMPC cases). Interestingly, a specific genomic signature of IMPC was defined by significantly more frequent gains of 8p12-q24 and 17q23-q24 regions, and losses located on chromosomes 8pter-p12, 13q12-q34 and 22q. Fifty-three regions of high level amplifications were found in 64% (16 /25 cases) of IMPC and IDC cases, respectively. Four regions of amplifications were preferentially observed in IMPC: 2 on chromosome 8 (8q12.1 and 8q21.13), one on chromosome 16p13.3 (*CCNF* gene) and one on chromosome 17q22 (*BCAS3* gene). Unsupervised analysis of transcriptomic data showed that IMPC and LVI+ N+ IDC clustered in groups according to their genomic status rather than according to their histological type. However, supervised analyses of transcriptomic data from the IMPC and LVI+ N+ IDC showed that genes involved in cell-matrix adhesion or cytoskeleton organization were differentially expressed between the two groups.
Conclusions: IMPC are luminal carcinomas characterized by recurrent amplicons (8q, 16p and 17q) and a genomic signature associating 8p losses/8q gains and 16p gains/16q losses combination with 17q23-24 gains, 13q and 22q losses. Transcriptomic analyses identified genes of interest involved in cell-matrix adhesion and cytoskeleton organization that could lead to the identification of new therapeutic targets in IMPC.

186 Fhit, Wwox and AP2γ Expression Levels Correlate with Basal Phenotype in Breast Cancer
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Background: Expression of Fhit and Wwox proteins, tumor suppressors encoded by fragile loci *FRA3B* and *FRA16D*, are concordantly lost in breast cancers. The current study examined correlations among Fhit, Wwox, transcription factors AP2α and AP2γ, cytokeratins 5/6 (CK5/6), epidermal growth factor receptor (EGFR), estrogen receptor (ER), progesterone receptor (PR), Her2 and their associations with breast cancer phenotypes.
Design: Tissue microarrays constructed from 837 breast cancer blocks were immunostained. Expression in >10% of tumor cells was considered positive for cytoplasmic CK5/6, membranous EGFR, nuclear AP2α and AP2γ. Cytoplasmic Fhit and Wwox staining was scored according to staining intensity. ER, PR and Her2 status of tumors was from records. Correlations among immunohistochemical markers and tumor subtypes were assessed by univariate and multivariate statistical methods.
Results: Triple negative tumors showed more frequent expression of EGFR, CK5/6 (p<0.001) and AP2γ (p = 0.003) and more frequent loss of Fhit and Wwox (p<0.001), with inverse correlation between Fhit, Wwox and EGFR ER, PR expression (p<0.001). Reduced Fhit expression was more common in Her2 and AP2γ positive cases (p<0.001, p=0.002). There was direct correlation between Fhit and Wwox (p<0.001) and a borderline positive relation between AP2γ and α (p=0.054).
Conclusions: Results suggest that reduced Fhit, Wwox and nuclear AP2γ expression have roles in pathogenesis of basal-like differentiation in breast cancer. Alteration of expression of fragile site genes occurs in most of these cancers and may contribute to defects in DNA repair, as observed in BRCA1-deficient cancers. Thus DNA damage response checkpoint proteins could be targets for treatment.

187 Expression of the Epithelial Mesenchymal Transition Regulator Snail in Matrix-Producing Carcinomas of the Breast
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Background: Epithelial mesenchymal transition (EMT) is defined as the dynamic transition from polarized, immotile epithelia to highly mobile, mesenchymal-like cells. EMT is hypothesized to promote metastasis of carcinomas, and is ultimately regulated by transcription factors such as Snail that downregulate epithelial and upregulate mesenchymal genes. The zinc-finger transcription factor Snail particularly functions in repression of E-Cadherin expression and cell migration. EMT is accompanied by a morphologic switch, as the polarized epithelial cells become more spindled and mesenchyme-like. However, EMT is thought to occur only in a small percentage of tumor cells at any one time. Therefore we hypothesized that matrix-producing carcinomas (MPC) of the breast, which reveal a mixed epithelial and mesenchymal component with a chondromyxoid matrix, may show EMT and increased expression of Snail throughout both tumor components.
Design: Archival paraffin embedded material of 12 MPCs and 1 lymph node metastases were examined by IHC for the expression and localization of Snail. Staining was evaluated for positivity in both the epithelial and chondroid metaplastic component. A minimum of 5% staining of the total tumor cell population was required for a positive result.
Results: Overall, 92% of the study cases revealed simultaneous nuclear and cytoplasmic positive staining in the conventional and metaplastic carcinoma component. Those tumor cells with metaplastic features were more likely to express nuclear staining, while the conventional carcinoma cells had a stronger correlation with cytoplasmic staining.

Snail expression in tumor cells			
Location	Conventional CA	Metaplastic CA	LN
Nuclear	4.4%	34%	12%
Cytoplasmic	95.6%	66%	88%

CA=Carcinoma component, LN= lymph node
Snail was not expressed in normal mammary tissue.
Conclusions: EMT is hypothesized to allow epithelial-derived tumors to acquire a more aggressive phenotype through the loss of epithelial cell polarity. During embryogenesis, Snail is involved in gastrulation and neural-crest migration, while in tumor progression, Snail has been observed to repress E-cadherin expression, allowing the acquisition of invasive and migratory properties that are critical for metastasis of carcinoma cells. Our findings suggest that the high expression rate of Snail in MPC may have an important function in EMT. The strong nuclear expression in the metaplastic component may play a role in the known high metastatic potential and aggressive behavior of MPC.

188 Ultrasound-Guided Needle Core Biopsy of the Axilla Often Samples Sentinel Node
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Background: Axillary lymph node status is an important prognostic factor in the assessment of breast cancer patients. Sentinel lymph node biopsy is the standard of care for screening clinically-node negative patients. More recently, axillary ultrasound has proven to be a valuable technique for screening the axilla. At our institution, a negative biopsy is followed up with a sentinel node biopsy to ensure adequate screening of the axilla. Our study aims to determine how often a negative ultrasound guided lymph node biopsy samples sentinel lymph node.
Design: 54 patients with negative axillary lymph node biopsies were identified from the University of Chicago Pathology archives (2005 - 2008). Sentinel node excision, along with non-sentinel nodes when available, were reviewed to determine which nodes exhibited biopsy site changes.
Results: All 54 patients with negative ultrasound-guided needle core biopsy underwent sentinel node biopsy (average 3 nodes removed; range 1-10) and 22 had additional non-sentinel nodes excised (average 1, range 1-3) in the vicinity of the sentinel node (taken due to accessibility at the time of biopsy). Eight of the 54 patient had a positive sentinel node on frozen section resulting in complete axillary dissection (average 16 additional nodes recovered). Changes consistent with prior ultrasound biopsy were found in a sentinel node in 25 patients (46% of all cases reviewed); in a non-sentinel node in 5 patients (9%) and in a node present in axillary dissection in 3 patients (6%). In 21 cases, a prior biopsy site was not identified and was presumed to be left behind in the remaining axillary nodes in the patient. When present, the biopsy site exhibited features consistent with the time elapsed since the ultrasound-guided procedure. Interestingly, of the eight positive sentinel nodes on frozen section, four exhibited biopsy site changes that were away from the focus of metastatic carcinoma.
Conclusions: Negative ultrasound-guided needle core biopsy often samples one of the sentinel nodes (46% of cases). The particular node with which biopsy site change is related should be noted in pathology reports. This vital information will help radiologists further refine biopsy thresholds and criteria.

189 Intraoperative Evaluation of Axillary Sentinel Lymph Nodes Using Touch Imprint Cytology and Rapid Immunohistochemistry
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Background: Haematoxylin and eosin stained frozen sections (FS) are traditionally used for the intraoperative evaluation of sentinel axillary lymph nodes. The aim of our study was to compare FS with touch imprint cytology (TIC) and ultra rapid immunohistochemistry (IHC) as intraoperative diagnostic tool.
Design: TIC and ultra rapid IHC (Choi et-al Jpn J Clin Oncol 2006) were performed on 62 consecutive cases of fresh axillary sentinel lymph nodes biopsies and compared with FS. Permenant Paraffin section H&E diagnosis was taken as gold standard. TIC smears were prepared from every corresponding tissue submitted for frozen section. Ultra rapid IHC (CK AE1/AE3) took 25 min and was performed at the same time.
Results: Final diagnosis on paraffin section showed 27 cases with axillary sentinel node metastasis, including 6 micrometastasis. The frozen section H&E detected 26(96.3%) positive lymph nodes. 1 case of micrometastasis was missed on FS. TIC detected 21(77.7%) metastasis; 6 metastatic tumors were missed including 5 micrometastasis. 1 case of metastatic carcinoma was missed due to poor smear technique. IHC detected 25(92.6%) metastasis, 2 metastatic deposits failed to pick the immunostain, however, all cases of micrometastases were positive. Final results are shown in table 1.

RESULTS			
	TOUCH IMPRINT	IHC	FROZEN
SENSITIVITY	77.77%	88.88%	96.29%
SPECIFICITY	100%	97.1%%	100%
POSITIVE PREDICTIVE VALUE	100%	96%	100%
NEGATIVE PREDICTIVE VALUE	85.36%	91.89%	97.22%
ACCURACY	90.31%	93.54%	98.38%

Conclusions: Our study shows that frozen section H&E remains superior to TIC & ultra rapid IHC in detecting axillary sentinel node metastases. TIC missed 5 out of 6 (83.3%) micrometastasis and should not be considered a sole diagnostic technique for intraoperative diagnosis. Ultra rapid IHC is best at detecting micrometastasis, however, the procedure requires technical expertise.

190 Papillary Carcinoma of the Breast (PC-B) Lacks Evidence of RET Rearrangements Despite Morphological Similarities to Papillary Thyroid Carcinoma (PTC)

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Background: PC-B can occasionally display nuclear features similar to those seen in PTC. In addition, there are a few reports describing primary breast carcinomas resembling the tall-cell variant of PTC. The aim of this study was to evaluate a series of PC-B for the prevalence of the histologic features that mimic those of PTC and to access whether these reflect the presence of the RET chromosomal rearrangements characteristic of PTC.

Design: A series of 33 intraductal/intracystic PC-B with or without associated invasive carcinoma was histologically reviewed to confirm the diagnosis and evaluate for the presence or absence of morphological features of PTC including the characteristic nuclear features (nuclear overlap, grooves, clearing, and inclusions) and cytological features of the tall-cell or columnar variants. RET rearrangements were studied in a subset of these cases by FISH and RT-PCR. Paired probes localizing to the centromeric and telomeric ends of the RET gene on chromosome 10 were developed from human BAC clones and used in a break-apart FISH approach. These probes were validated in a cell line harboring the RET-PTC1 rearrangement and in 2 cases of PTC (where split green and red signals were identified in 42-45% of nuclei) and in 17 normal breast and thyroid control tissues (in which split signals were seen in only 1-3% of nuclei). Published RT-PCR primers designed to detect RET-PTC1, RET-PTC2, and RET-PTC3 fusions were used in both single round and nested PCR approaches.

Results: Nuclear overlap, grooves, and clearing were at least focally identified in 23 (70%), 12 (36%), and 9 (27%) cases, respectively, while none of the cases displayed intranuclear inclusions. Cytological features resembling the tall-cell or columnar variants of PTC were focally identified in 1 (3%) and 2 (6%) cases, respectively. Four of 19 tested cases displayed split FISH signals in a low percentage of cells (range: 9-11%), and were considered equivocal for RET rearrangement. These 4 cases, as well as another 15 cases with amplifiable RNA (out of 22 tested cases) were all negative for the 3 RET-PTC fusions evaluated by RT-PCR.

Conclusions: Although PC-B can show, at least focally, cytologic and nuclear features reminiscent of PTC, there is no evidence that they share the most common RET-PT rearrangements characteristic of PTC.

191 Estrogen Receptor Status Is Generally Stable during Disease Progression from Primary to Metastatic Breast Cancer

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Background: Accurate assessment of ER status is necessary prior to endocrine therapy for breast carcinoma. Although ER is usually determined on newly diagnosed breast carcinomas, determination of ER status on metastatic breast carcinomas is often requested by oncologists. Whether ER status can change during disease progression or after chemotherapy or hormonal therapy remains controversial.

Design: Breast carcinoma samples from 56 women with known ER status in both primary tumors and paired metastases (30 locoregional and 26 distant metastases) were retrospectively reviewed. Before their metastatic lesions were sampled, 44(79%) patients underwent chemotherapy and 30 (54%) patients received adjuvant hormonal therapy. ER status was measured by immunoperoxidase study either in tissue sections (50 primary tumors and 10 metastatic tumors) or in fine-needle aspiration smears (6 primary tumors and 46 metastatic tumors). ER status was defined as positive if ≥10% tumor cells demonstrated nuclear staining.

Results: The interval between the diagnosis of the primary tumor and sampling of the paired metastatic tumor ranged between 0-216 months. ER status of primary and metastatic tumors agreed in 52 of the 56 (93%) patients, including 37 (71%) positive tumors and 15 (29%) negative tumors. A discrepancy of ER status was observed in 4 patients, with two having positive status in primary/negative status in metastatic tumors and another 2 having negative status in primary/positive status in metastatic tumors. In 3 of the 4 patients, the ER score of the positivity were 10% (marginally positive). All the metastatic tumors where ER was tested in the four patients were found in locoregional lymph nodes.

Conclusions: ER status in breast carcinoma is generally stable during disease progression. Chemotherapy and/or hormonal therapy do not significantly affect ER status in metastatic diseases.

192 Comparative Analysis of p53 Expression in Triple Negative Versus Non-Triple Negative Invasive Breast Cancers

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Background: Triple negative (TN) breast cancers characterized by negativity for estrogen receptors (ER), progesterone receptors (PR) and Her2, lack the benefit of specific therapy that targets these proteins and are associated with shorter survival and a higher recurrence rate. To improve the prognosis of TN tumors, a new effective therapeutic strategy is needed. Recent studies suggested that p53 status might have a different predictive value for efficacy of anthracycline/alkylating agents- based chemotherapy regimen between TN and non-TN breast cancers. Therefore, we compared expression of p53 between TN and non-TN tumors in patients with grade II or grade III invasive ductal carcinoma.

Design: Clinical characterizes and tumor profiles were analyzed in 58 patients (24 TN tumors and 34 non-TN tumors). Of the 34 non-TN tumors, 18% were ER-PR-Her2+, and 82% were ER+ or PR+ or both ER+ PR+. The expression of p53 was compared between the TN and non-TN groups by one-way ANOVA with post hoc test, and also examined in core-needle biopsies and subsequent resection specimens.

Results: The TN tumors were larger than the non-TN tumors in size (p<0.03), and appeared to have a higher rate of axillary nodal metastasis than the non-TN tumors

(75% vs. 68%, p=0.57). The expression of p53 in TN group was significantly higher than that in the non-TN group (p<0.0001). Moreover, the p53 expression in the TN tumors was significantly increased compared to the non-TN tumors that were ER+ or PR+ or both ER+PR+ (p<0.001), while there was no significant difference of the expression of p53 between the TN tumors and the non-TN tumors that were ER-PR-Her2+. Tumors showed a similar expression of p53 between core biopsies and resection specimens on the same patients in both the TN and non-TN groups.

Conclusions: Our results indicate that TN breast tumors are associated with a higher expression of p53 compared to non-TN tumors, which may contribute to TN tumors having a poorer prognosis. Hormonal receptor status rather than Her2 status appears to play an important role in significantly increased p53 expression in TN tumors. The expression of p53 in core-needle biopsies can be predictive of its status in resection specimens in both the TN and non-TN tumors.

193 Molecular Characterization of DCIS by Immunohistochemistry: Correlation with Histologic Grade, Ki-67, Bcl-2 and p53 Expression

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Background: DCIS is a heterogenous disease with a wide spectrum of morphologic types. Recently, gene expression studies on invasive carcinoma have revealed molecular subtypes. Immunohistochemical analysis using ER, PR and Her2Neu as a surrogate for gene expression, invasive carcinomas have been classified into four major subtypes: luminal A (ER+, PR+, Her2-); luminal B (ER+, PR+, Her2+); Her2 + and triple negative (ER-, PR- and Her2-) . The aim of this study is to characterize DCIS into distinct molecular subtypes by immunohistochemical stains for ER, PR and Her2Neu. To determine if there is a correlation of the different groups of DCIS with histologic grade, ki-67, Bcl-2 and p53 expression.

Design: A total of 118 patients with a diagnosis of pure DCIS were retrieved from the archives of the Department of Pathology at UTSW Medical Center, from January 2000 to August 2007. Clinical and pathologic data such as patient's age, histologic grade and results of immunohistochemical studies for ER, PR, Her2Neu, ki67, bcl2 and p53 were retrospectively analyzed. Histologic grading of DCIS was performed according to CAP guidelines. DCIS was stratified into four groups: (ER+, PR+ and Her2-); (ER+, PR+ and Her2+); (ER-, PR-, Her2+) and (ER-, PR- and Her2-). Each of the four groups of DCIS was correlated with histologic grade, Ki-67, Bcl2 and p53 expression. Statistical analysis was performed by one-way ANOVA with post hoc test.

Results:

Correlation of DCIS with age, nuclear grade, Ki-67, Bcl-2 and p53 is shown below: (mean ±SEM)

DCIS subtypes Total =118	ER+PR±Her2- 75 (63.6%)	ER+PR±Her2+ 22 (18.6%)	ER-PR-Her2+ 14 (11.9%)	ER-PR-Her2- 7 (6.0%)	P Value
Age	61.8±1.44	60.41±1.94	62.9±2.36	59.1±2.85	0.168
Low-grade DCIS	19.1%	16.7%	0%	0%	
Intermediate grade DCIS	50%	20.8%	14.3%	21.2%	
High grade DCIS	30.9%	62.5%	85.7%	78.8%	0.002
Bcl-2	78.9±4.52	49.6±12.4	6.25±4.13	19.0±14.2	<0.001
Ki-67	14.4±1.32	20.4±3.00	36.4±12.2	33.6±4.87	<0.001
P53	11.2±2.62	11.2±4.81	49.0±16.4	24.4±8.50	<0.001

Conclusions: Low and intermediate grade DCIS were predominantly of the luminal A and B types. Both were associated with high Bcl-2 expression, low proliferation and low p53. Triple negative and Her2Neu subtypes were significantly associated with high grade DCIS, increased p53 expression, high proliferation and low Bcl-2.

194 Positive HER2 Immunoreactivity in DCIS Is Associated with a Higher Incidence of Invasive Disease, Particularly in the Subsequent Resection in a Group of Patients without Radiographic Evidence of Invasion Disease

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Background: Prognostic significance of ER, PR and HER2 phenotype has been established in invasive carcinoma. The implication of these phenotypic patterns in DCIS remains uncertain. This study is to determine whether DCIS ER/PR/HER2 phenotypes have any role in progression to or association with invasive cancer in DCIS.

Design: Clinical and histologic materials of 104 patients, whose DCIS were tested for ER, PR and HER2, were reviewed. No invasive disease was suspected in radiographic evaluation. All patients underwent biopsy and subsequent resection. The HER2 status was determined by HercepTest and considered positive when 2+ >10% was detected. The rate of invasive disease was evaluated in relation to various histopathologic features of DCIS as well as their ER/PR/HER2 status.

Results: Invasive disease was identified histologically in 23 patients (22%), 3 in initial biopsy (size 1-2 mm each) and 20 more in the subsequent resection (size 1-15 mm, mean 45 mm). The phenotype of all DCIS and those with invasive disease are summarized in Table 1. HER2 positive DCIS (HER2+ and luminal B) have significantly higher rate of invasive disease than HER2 negative DCIS (15/36, 42% vs. 8/68, 12%; p=0.002). The rates of invasion associated with luminal B (50%) and HER2+ phenotypes (36%) were significantly higher than that associated with the luminal A DCIS (11%) (p<0.001 and p=0.005, respectively).

Table 1	Lumnal A	Her2+	Luminal B	Basal
DCIS	62/104 (60%)	22/104 (21%)	14 (3%)	6 (6%)
Invasive disease	7/62 (11%)	8/22 (36%)	7/14 (50%)	1/6 (17%)

Conclusions: Invasive disease was identified in 22% of the patients who had DCIS and no radiographic evidence of invasive disease. Majority of these invasive lesions were associated with HER2+ DCIS, and were small (mean <5 mm) with only 13% of them detectable in the initial biopsy. These observations suggest a possible role of HER2 in the progression of in-situ to invasion. HER2 test might predict a group of DCIS with

higher risk of invasive lesion in resection sepcimen. Therapies targeting HER2 may be used in cancer prevention in patients with DCIS.

195   Tissue Microarray (TMA)-Based IHC Study Can Significantly Underestimate the Expression of HER2

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Background: The use of TMA has been shown to be a very cost effective tool in translational research. The accuracy of TMA-based studies is largely dependent on the uniformity of the staining pattern for a given antibody, which is frequently not the case for breast markers. This project aimed to study the non-concordance rates of ER, PR and HER2 expression in TMA and whole sections of breast cancer.

Design: Seventy-five cases of pure DCIS (ductal caicinoma in situ, 0.5cm - 4.8cm in size) were compared for the expression of ER, PR and HER2 in TMA and whole sections. The TMA was constructed with 3 cores (1mm) per case. ER and PR were recorded as Allred scores with 3 and above as positive, 2 and under as negative. HER2 were recorded as positive if >30% of tumor cells showed 3+ membrane staining. Concordance was defined as the same staining result for one or more cores from TMA with whole section result, and non-concordance includes cases with opposite staining result in at least one of the three cores compared to whole section (dis-concordance) and non-informative defined as no tissue or no tumor cell present in all three cores of a given case.

Results: Among the 75 DCIS cases, the rates of under estimation for ER, PR and HER2 expression were 10% (P=0.491), 24% (P=0.101), and 73% (P=0.001), respectively. The non-concordant rates (including discordant and non-informative cases) were 10.7%, 24%, and 28% for ER, PR and HER2, respectively (p=0.012). The non-concordant rates were inversely related to core number, with 46.67%, 22.67%, and 11.56% for one core, two cores, and three cores per marker per case (p<0.001). However, the non-concordant rates were not associated with tumor sizes.

Conclusions: While TMA's have been shown to be an effective tool for translational research, the current study calls attention to potential concerns for the evaluation of markers that do not demonstrate uniform expression throughout tumors. Significant under estimation and non-concordance by TMA analysis for breast cancer warrants caution in the interpretation of results. Increased numbers of tissue cores per case may help to improve the accuracy and concordance rates with whole sections for such studies.

196   A Glimpse into Tumor Metabolism by Magnetic Resonance Spectroscopy: Choline Compounds and Choline Kinase Gene Expression in Human Breast Cancer

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Background: Magnetic resonance (MR) imaging of the breast is a highly sensitive technique with increasing importance in the diagnosis of breast cancer, especially when multifocality is suspected or the tumor is not visible on mammography. However, tissue biopsies to fully characterize the MR-detected abnormalities are often necessary. MR spectroscopy is a further development that allows functional *in vivo* analysis of the tissue by assessing tissue metabolism. Prior cell-line studies indicated that the metabolic pathway catalyzed by the enzyme choline kinase-α (CHKα) is increased in malignancies. By studying human breast cancer tissue, we wish to correlate MR spectroscopy of the metabolite choline with molecular analysis of CHKα and histopathological features.

Design: Fresh tissue samples of 5 invasive ductal carcinomas (IDC) and 5 invasive lobular carcinomas (ILC) were examined by MR spectroscopy with measurements of choline compounds. Molecular analysis of CHKα mRNA was performed by real time PCR (qPCR) after laser capture microdissection and RNA extraction of malignant epithelial cells. Adjacent normal tissue served as matched control. Correlations between cholines, the corresponding CHKα mRNA levels and histopathological tumor types were evaluated with standard statistical software.

Results: Higher expressions of CHKα mRNA are associated with lower values of choline compounds in both IDC and ILC tumor samples (IDC: r^2=0.91, p<0.012; ILC r^2=0.78, p<0.047). Increased CHKα mRNA levels in ILC result in more than 2.6-time reductions in choline compounds when compared with IDC. Matched benign tissue did not show decreased choline or increased CHKα mRNA levels.

Conclusions: We show that CHKα levels are inversely correlated with choline intensity in representative human breast cancer samples. Our results indicate that MR spectroscopy may differentiate malignant from benign tissue. Based on metabolomic characteristics, it may even be possible to distinguish morphologic subtypes of breast cancer such as lobular or ductal types. Further studies of the complete choline pathways will advance the understanding of both metabolic characteristics in human breast cancer and their features in MR spectroscopy.

197   Hispanic Women Are More Likely To Have HER2 Positive Tumors When Compared to Non-Hispanic White women

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Background: Hispanic women are at a lower risk of getting breast cancer compared to non-Hispanic White (NHW) women, yet they experience a higher risk of mortality after diagnosis. There is some evidence to suggest that there are pathologic differences when comparing these populations; however, limited research has been done on Hispanic populations.

Design: We reviewed pathology reports, obtained paraffin blocks of breast cancer tissue and established tissue microarrays from NHW and Hispanic women (n=285) who were Colorado participants in the 4-Corners Breast Cancer Study, a population-based, case-control study of breast cancer designed to investigate factors that contribute to ethnic

disparities. Using this TMA collection, we evaluated the HER2 status of the invasive tumors. In addition, HER2 status was validated using conventional whole slides for a subset of these women. The CAP/ASCO guidelines were utilized.

Results: Our preliminary data indicate that there are differences in tumor pathology when comparing breast carcinomas from Hispanics and NHWs. In addition to having a higher prevalence of estrogen receptor negative tumors (30.2% versus 19.6%, respectively, P=0.03), Hispanics also had a significantly higher proportion of HER2 positive tumors compared to NHWs (37.5% versus 18.4%, respectively, P<0.01).

Conclusions: Given the disparities in breast cancer outcomes and tumor characteristics, it is highly plausible that breast cancers among Hispanic women comprise distinct tumor subtypes as compared with NHW women. These findings emphasize the importance of evaluating factors such as pathologic characteristics among different ethnic and racial populations in order to gain a better understanding of breast cancer development in these populations.

198   Intra-Operative Imprint Cytology of Sentinel Lymph Nodes in Breast Cancer. A Comparison with Conventional H & E and Computer Assisted Image Analysis

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Background: The intra-operative assessment of SLN by touch imprint cytology (TIC) is a reliable and accurate method for detection of metastasis. Because there is no loss of tissue, the SLN can be further analyzed by conventional H &E and cytokeratin immunohistochemistry. Automated computer-assisted detection of tumor metastasis by image analysis (IA) may assist in the identification of metastases.

Design: We retrospectively analyzed intra-operative TIC performed on 169 SLNs from 61 patients undergoing breast cancer surgery at UT southwestern Medical Center, Dallas. All SLNs were fixed in formalin and processed in a routine fashion. The protocol for the evaluation of SLNs consisted of an initial examination of permanent sections (first level) by H&E followed by three deeper levels and cytokeratin (AE1/AE3) immunostaining between each level. Recently, a computer assisted image analyzer was implemented in our laboratory for evaluation of SLN. This study compares TIC with conventional H&E and automated IA.

Results: There were 36/61 (59%) invasive ductal carcinomas, 7 (11.4%) invasive lobular, 6 (9.8%) mixed types, 4 (6.5%) mucinous, 8 (13.1%) DCIS. The average number of SLNs per case was 2.5. 23/169 (13.6%) were positive for metastases. Ten (5.8%) were positive by TIC, 18 (10.7%) by conventional H&E and 5 (3.0%) by IA. 9 of 10 (90%) positive SLNs by TIC were confirmed by H&E and one by IA. 159/169 (94%) of the SLNs were negative by TIC, of which 9 (5.7%) were positive by H&E and 4 (2.5%) positive by IA only. Sensitivity of TIC was 44%, a specificity of 100% a negative predictive value of 90.3% and a false negative rate of 5.3%. 83% of the macrometastasis were detected by TIC. 70% of the micrometastasis were detected by H&E.

Comparison of size of metastases and tumor by the three different methods.

No of SLN+ cases	TIC =10	H&E =9	I A =5	P anova
Size of metastases	7.51 mm	1.49 mm	0.56 mm	p<0.0001
Size of primary tumor	3.2 cm	3.37 cm	1.53 cm	p<0.4
ALND	7/10	5/9	0/5	

Conclusions: Touch imprint cytology of SLN was a fairly sensitive and highly specific method for detection of macrometastasis. There were no false positive. The size of the metastases showed an inverse relationship to the sensitivity of the detection method. The false negative results by TIC were due to the inability to detect micrometastasis. IA identified additional metastasis not detected by H&E and these tended to be isolated tumor cells.

199   Heparinase-1 Expression Is Progressively Increased with Increasing Stage in Non-Small Cell Lung Carcinoma and May Help Predict Recurrence in Early Stage Non-Small Cell Lung Cancer

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Background: Heparinase-1 (HPR1) is an endoglucuronidase that cleaves heparin sulfate proteoglycans, a main constituent in basement membrane (BM) and extracellular matrix (ECM). HPR1 promotes tumor metastasis by breaking down the BM and ECM. We studied HPR1 expression in non-small cell lung cancer (NSCLC) and its clinicopathologic significance.

Design: Paraffin-embedded surgical specimens from 54 patients with primary NSCLC were analyzed (37 adenocarcinomas and 17 squamous cell carcinomas). Twenty-eight specimens were stage I. Nineteen specimens were from patients with ipsilateral hilar node metastases--stage II disease. Seven specimens were from patients with locoregionally advanced stage III disease, 3 of which were treated with neoadjuvant chemotherapy/radiation. All specimens were stained for HPR1 expression (positive defined as >10% staining, with increasing intensity scored from 0 to 4 with 4 being the most intense).

Results: Twenty-one of 28 stage I specimens expressed HPR1 (mean intensity 1.62). Among these 21, recurrence occurred in 5 patients (24%). Among the 7 negative specimens, only 1 patient (14%) developed recurrence. Ten of 19 stage II specimens expressed HPR1 (mean intensity 1.92). Among these stage II specimens, recurrence occurred in 3 of the 10 patients with positive specimens (30%) and in none of the patients with negative specimens. Although not statistically significant, the disease-free survival for HPR1 positive tumors was lower than for HPR1 negative tumors (p=0.14). Three of 4 stage III specimens without neoadjuvant therapy were positive for HPR1 expression (mean intensity 2.3). The 3 stage III specimens with neoadjuvant therapy were negative. HPR1 staining intensity was not significantly different between stages, although the difference approached significance between non-neoadjuvant and neoadjuvant stage III specimens (p=0.6).

Conclusions: HPR1 expression appears to increase with increased stage, which may reveal the mechanism behind tumor metastasis. Neoadjuvant therapy may modify the role of HPR1 in metastases as evidenced by decreased expression in treated specimens. Recurrence appears to be higher in patients expressing HPR1. Patterns of recurrence were more erratic in stage III NSCLCs, but this may be due to patient heterogeneity and small sample size.

200 Expression of IMP3 in Primary and Metastatic Breast Ductal Adenocarcinomas

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Background: IMP3 is a RNA-binding protein and involved in multiple critical biological processes including RNA trafficking and stabilization, cell growth, and cell migration. We have shown that IMP3 is a novel molecular marker that predicts metastasis for renal cell carcinomas and urothelial carcinomas. The goal of this study is to compare IMP3 expression in primary and metastatic breast ductal carcinomas.

Design: 54 patients with breast ductal carcinomas were selected. They had surgery and follow-up at University of Massachusetts Medical Center. At the time of study, 24 cases were primary invasive ductal adenocarcinomas and 30 were metastatic ductal adenocarcinomas. Immunohistochemical stains were performed on paraffin-embedded tumor specimens using mouse monoclonal antibody specific for IMP3 (L523S, Corixa, Seattle, Wash) and our previously published protocols. The results were independently reviewed by two pathologists. Then, the association of IMP3 expression with patients' metastatic status was analyzed.

Results: In primary ductal adenocarcinomas, 2 out of 24 cases (8%) showed IMP3 overexpression while 14 out of 30 (48%) metastatic ductal adenocarcinomas had IMP3 overexpression (p<0.005). No IMP3 expression was found in adjacent benign tissues.

Table 1

	IMP3 +	IMP3 -
Primary tumor	2 (8%)	22 (92%)
Metastatic tumor	14 (48%)	16 (52%)

Conclusions: In contrast to normal breast tissue, we demonstrated that some breast ductal adenocarcinomas have high expression level of IMP3. The percentage of cases with such IMP3 expression increased 6 folds in metastatic carcinomas compared to the primary. These suggest that IMP3 is a potential prognostic biomarker to predict possible metastasis of breast ductal adenocarcinoma.

201 Does Estrogen Receptor Expression Vary with Fixation Time?

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Background: ER expression by IHC is said to vary with the time of fixation. However, only limited published data exist addressing this subject (Goldstein). In contrast to usual pathology practice, the tissue in that study was not processed immediately after formalin fixation. Instead the blocks were maintained in 100% cold ethanol prior to transfer to the tissue processor where there was an additional 90 minutes fixation in heated (40°C) 20% formalin prior to the dehydration steps.

Design: The goal of this pilot study is to examine 10 cases. Small pieces (4x4x2mm) 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 1 hr, 3 hrs, 6 hrs and 9-10 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 using a fast routine protocol for 2:30 hrs in a Shandon Excelsior tissue processor that did not include additional time in formalin. All blocks were then batch stained with rabbit antibody SP1 clone for estrogen receptor using the Ventana Benchmark™ XT automated processor. All cases were invasive carcinomas known to be estrogen receptor positive before inclusion in the study. Two pathologists reviewed every slide independently and the stains were evaluated as follows: Intensity of nuclear staining (1+, 2+, or 3+) and percentage of positive tumor cells.

Results: Five cases of invasive breast carcinoma have been analyzed thus far. All blocks, regardless of whether they were fixed for 1, 3, 6, or 9-10 hrs had strong and diffuse nuclear staining with ER. No significant staining difference was noted between the various fixation times.

Conclusions: Fixation times in 10% buffered formalin between 1 and 9 hrs do not affect ER expression. Further studies are needed to confirm this finding. Reference: Goldstein NS. Recommendations for improved standardization of immunohistochemistry. Appl Immunohistochem Mol Morphol 2007;15:124-133

202 Histopathologic and Staging Characteristics of ER/PR/HER2 "Triple-Positive" Mammary Carcinoma

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Background: Triple positive mammary carcinoma, i.e. ER (+), PR (+) and HER2 (+), represents a variant of luminal type B breast cancer (as defined by gene expression analysis). These tumors are less frequently encountered than the much celebrated "triple-negative" metaplastic basal-like carcinomas. As a group, have not been fully characterized.

Design: The Hartford Hospital Pathology database was queried for breast carcinomas from 2002-2007 whose ancillary studies showed an ER/PR/HER2 positive phenotype. Slides/pathology reports were reviewed. Clinicopathologic features were tabulated with regard to age, T-size, histologic type, nuclear/histologic grade, necrosis, lymphovascular invasion and N-stage. Ancillary studies were reviewed to confirm triple positive status. In two cases HER-2 FISH analysis was obtained.

Results: Fifteen "triple positive" cases were identified from the files, accounting for 0.5% of the 2765 breast carcinomas diagnosed from 2002-2007. The abstracted clinicopathologic features are presented below. Histologically most cases showed classical high grade "schirrous" ductal carcinoma (NOS) including pleomorphic nuclei, prominent desmoplasia, modest inflammatory round cell response and virtually no tubule formation. There were two exceptions to this generalization. Cases #4 and #6 were invasive lobular carcinomas (classical and pleomorphic types, respectively). HER2 positivity was confirmed with FISH analysis for both cases

CASES OF TRIPLE POSITIVE MAMMARY CARCINOMA (N=15)

Case	Age	T-size	Hist Type	HG/NG	Necrosis	LVI	LN-stage
1	38	2.5cm	ductal	III/III	present	absent	1/4(mic)
2	71	1.4cm	ductal	III/II-III	present	absent	1/9(mic)
3	60	1.9cm	ductal	III/III	present	absent	0/2(i+)
4	51	4.8cm	lobular*	NA/I	absent	absent	1/2(i+)
5	43	2.3cm	ductal	III/II-III	present	absent	3/10
6	65	3.3cm	lobular*	NA/II&III	present	present	0/3
7	35	1.2cm	ductal	III/III	absent	absent	4/9
8	53	3.0cm	ductal	III/III	absent	absent	2/13
9	44	3.5cm	ductal	III/III	absent	present	0/15
10	36	2.0cm	ductal	III/III	present	absent	1/1
11	56	2.7cm	ductal	III/III	present	absent	1/14
12	65	2.2cm	ductal	III/III	present	absent	0/1
13	51	1.1cm	ductal	III/III	present	absent	0/2
14	39	5.0cm	ductal	III/III	absent	absent	0/2
15	45	0.5cm	ductal	II/II	present	absent	0/1

*FISH was (+) in both cases of lobular carcinoma.

Conclusions: There is a characteristic histology associated with ER/PR/HER2 (+) status. These tumors, however, are not homogeneous and include both ductal and lobular variants.

203 Visualizing Internal Structures of the Breast in Thick-Sections Using X-ray Dark-Field Imaging

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Background: X-ray dark field imaging (XDFI) using synchrotron light and Laue geometry analyzer is a new imaging system that makes it possible to extract only refracted components of x-ray (Ando et al. 2001 Jpn. J. Appl. Phys). The new method attains extremely high contrast (approximately 100:1 than the absorption contrast) and high resolution (about 20 microns) without contrast material. The aim of this study is to validate XDFI by comparing its images of various breast lesions with those of the stained histological sections.

Design: Paraffin embeded blocks (3-4mm in thickness) containing normal terminal duct lobular units (TDLU), cysts, nodular adenosis, phyllodes tumor, non-invasive and invasive carcinoma were visualized using XDFI at beamline BL14 of the Photon Factory in Tsukuba, Japan. The images were compared with the images of the hematoxylin stained thick section as well as hematoxylin and eosin stained thin section.

Results: Although the most lesions were located within the dense breast, XDFI successfully visualized them. Nodular adenosis was seen as a high refractive arborescent area. The refractivity of the nodular adenosis is slightly greater than those of the surrounding normal TDLU (Figure 1a, arrow).
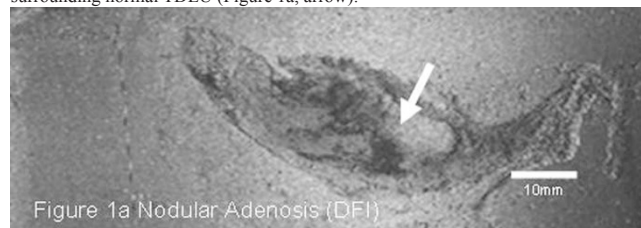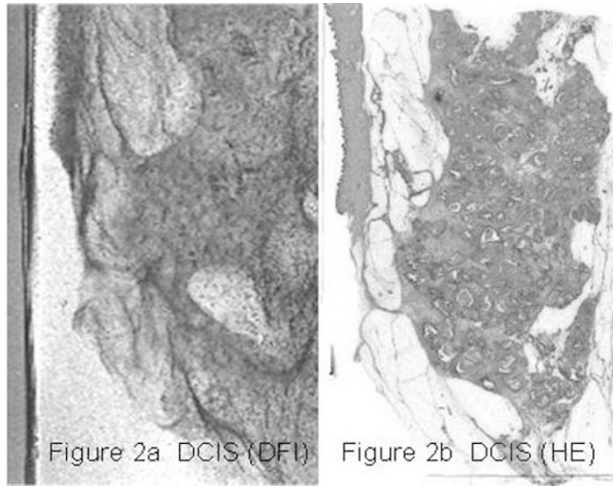

Figure 1a Nodular Adenosis (DFI)

Figure 1b Nodualr Adenosis (HE)

High grade DCIS was observed as multiple pore like areas less refractive than the surrounding fibroglandular tissue (Figure 2a).

Figure 2a DCIS (DFI)   Figure 2b DCIS (HE)

These XDFI images closely simulated those of conventional thick and thin sections (Figure 1b,2b).

Conclusions: XDFI is a powerful tool to visualize internal structures of the dense breast without either destruction or contrast material.

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204   The Effects of ASCO/CAP Guidelines on Her2/Neu Reporting: An Analysis of 662 Consecutive Breast Cancer Cases with Emphasis on IHC 2+ and 3+ Cases
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Background: New guidelines were recently published by ASCO/CAP for evaluating Her2 protein expression and gene amplification in invasive breast carcinoma. By FISH, a Her2/Neu:CEP17 ratio greater than 2.2 is considered a positive result.By immunohistochemistry (IHC), a positive result (3+) is given when there is a complete intense membranous Her-2 labeling in at least 30% of the tumor cells. Prior guidelines (FDA approved) required at least 10% of tumor labeling by IHC to call the tumor Her-2 positive (3+) . The higher percentage of tumor staining by IHC (30% instead of 10%) was recommended based on the experience of the panelists rather than published data.

Design: We have reviewed the Her-2 results of 662 consecutive breast carcinoma cases processed at our institution. The results on all cases were reported according to the ASCO/CAP guidelines. We rescored the cases using the original FDA approved guidelines. We compared the results of the old and the new guidelines with an emphasis on the 2+ and 3+ cases by IHC. FISH results were available on all IHC 2+, the majority of 3+ and a subset of 1+ and 0 cases.

Results: The cases with a score of 0 or 1+ were the same using both guidelines (112 and 295 respectively). Of those, FISH was done on twelve Her2/neu 0+ cases (all FISH negative) and on 44 Her-2/neu 1+ cases (5 borderline, 39 FISH negative). For the IHC 2+ and 3+ cases, the results are summarized in table-1. Fourteen cases that scored as 3+ using the old guidelines were scored as 2+ according to the new guidelines. Of these 14 cases, six were FISH positive, seven were FISH negative and one was borderline.

Table-1: Her-2 IHC scoring using the old and new guidelines

IHC score	N	FISH +	FISH borderline	FISH -
2+ (new)	191	27/187	11/187	149/187
2+ (old)	177	20/172	10/172	142/172
3+ (new)	64	50/51	1/51	0/51
3+ (old)	78	56/65	2/65	7/65

Conclusions: Our data shows that 18% of the cases scored as 3+ by IHC using the old guidelines are scored as 2+ using the new ASCO/CAP guidelines. Half of these cases are Her-2 negative by FISH. Therfore the use of a higher cut-off (30% as compared to 10%) for defining the positive 3+ cases by IHC seems to be justified.

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205   HER2 Rabbit Monoclonal Antibody 4B5 and Silver SISH: High Performance in Surgical Pathology for Appropriate Patient Care
H Itoh, N Kato, A Serizawa, T Itoh, S Umemura, RY Osamura. Tokai University School of Medicine, Isehara, Japan.

Background: For the daily HER2 testing, ASCO/CAP guideline requires to retest if the results of either IHC and FISH are "equivocal". It has been reported that rabbit monoclonal antibody 4B5 provides excellent sensitivity, specificity, and interlaboratory reproducibility. In addition, non-fluorescent detection of HER2 gene copies, silver SISH has been recommended for routine laboratory use. This study is aimed at to elucidate whether the combination of HER2 IHC by 4B5 and HER2 SISH functions efficiently for routine HER2 testing.

Design: Tissues from 44 cases of breast cancers were subjected to the following IHC, FISH and silver SISH studies. Rabbit monoclonal antibody 4B5 was used for automated immunohistochemistry(IHC) on formalin-fixed paraffin ebedded(FFPE) sections. Interpretation was done by following ASCO/CAP guideline. Silver SISH was also performed on the consecutive sections of the same cases for HER2. Immunohistochemistry by monoclonal antibody CB11 was also done for comparison. Silver SISH resulted in countable gene copies for HER2 and CEP17. Ratio HER2/CEP17 was calculated and compared with HER2 FISH(Abbott Moleculer Inc.).4B5,CB11 & SISH were supplied from Ventana Medical Systems, Inc.

Results: For IHC and FISH, 19 cases showed 4B5+ FISH+ and 14 cases revealed 4B5- FISH-. SISH and FISH also showed excellent concordance except for one FISH+

case which was SISH-. Eight cases of 4B5 2+ were also CB11 2+, and the discordance between two antibodies were two cases, less cases of HER2 2+ by 4B5. Seven of eight 4B5 2+ case were FISH negative, one 4B5 2+ case was FISH equivocal. One 4B5 2+ cases was FISH- but SISH+.

Conclusions: Our studies suggest the combination of 4B5 and SISH could serve as the appropriate target detection system for the efficient patient care by Trastuzumab for advanced and early breast cancers.

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206   Benign Mucocele like Lesions of the Breast: Revisited
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Background: Mucocele like lesions (MLL) of the breast are ruptured cystically dilated mucin producing ducts that discharge their contents into the stroma.They constitute a spectrum of changes from benign to atypical to malignant. The current management of these lesions diagnosed on core biopsy (CB) is excision. The goal of our study was to evaluate the necessity of this practice for benign MLL (BMLL).

Design: Retrospective review of the pathology database from 1/1/2000 to 6/1/2008 identified 61 cases, with follow up information available in 50. Clinical, radiologic and pathologic information was correlated. CBs were reviewed to confirm the diagnosis of BMLL and exclude atypical or malignant changes. Excisions were also reviewed to determine final diagnosis and verify previous CB changes.

Results: 45 patients underwent surgery while 5 patients were followed >1year and are stable. Patients' age ranged from 44 to 76 years (mean=54.6). Method of diagnosis was stereotactic CB for calcifications (Ca++) in 44 and ultrasound for masses in 6. Ca++ on mammogram were clustered(35 cases), new(3), coarse(2) linear(1), granular(1), pleomorphic(1) and suspicious(1). The size of the BMLL on CB ranged from incipient lesions (<0.1 cm) to 0.6cm. Most excisions had no residual MLL (36/45=80%). In 7 cases (15.6%) atypical duct hyperplasia (ADH) was present, 4 with residual MLL. In 1 of these cases, the residual MLL showed a continuum from florid duct hyperplasia to ADH at the CB site. The other 6 cases showed ADH adjacent to but not at the CB site. The size of the BMLL ranged from incipient to 0.5cm. Ca++ were identified in the BMLL in 6 cases. In the 7th case, Ca++ were identified only in fibrocystic changes while the BMLL was an incidental finding (0.1cm). There was only 1 case of DCIS in which the Ca++ were suspicious and widespread. Review of CB showed BMLL (0.3cm) and atypical lobular hyperplasia. Excison showed residual MLL with ADH and LCIS admixed with focal low grade DCIS.

Conclusions: Clinically, BMLL ocurr in older women. In this series, the largest of its kind, the upstage rate of BMLL diagnosed on CB was 17.8%. Most BMLL diagnosed on CB lacked a residual component on excision. With the exception of the DCIS case, radiologic features were not predictive of atypia. The size of the BMLL on CB did not affect outcome as both incipient and incidental lesions were associated with ADH. In most cases, the ADH was adjacent to but not at the CB site. Thus, due to associated ADH, sampling reasons, and intralesional heterogeneity, we continue to recommend excision of BMLL diagnosed on CB.

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207   Spindle Cell (Sarcomatous) Carcinoma of the Breast: Immunohistochemical Profile and the Clinical Outcome
S Johnykutty, D Hicks, K Skinner, C Andrus, L Schiffhauer, M Worman, X Wang. U of Rochester, Rochester.

Background: Spindle cell carcinoma is in the differential diagnosis for spindle cell lesions of the breast. While the basal-myoepithelial markers have been used as a tool for diagnosis, their consistency is uncertain. In addition, there is contraversy regarding the morphological grade of this lesion in regards to clinical outcome.

Design: Cases for study were identified using the following criterial: 1) spindle cells mixed with malignant epithelial component, and/or 2) at least one of the basal-myoepithelial marker showing focal expression in spindle tumor cells. Immunohistochemical stain for pan-cytokeratin, CK5/6, CK14, p63, and EGFR were performed. Clinical follow up was obtained by reviewing the charts.

Results:

tumor characteristics and outcome

case#	histologic type	tumor grade	tumor size (cm)	follow up interval (month)	status
1	mixed	high	1.5	25	died of metastasis of epithelial component
2	mixed	high	3.2	60	DF
3	mixed	high	ND	48	DF
4	spindle	low	3.0	53	DF (locally recurred)
5	spindle	low	2.0	36	DF
6	spindle	low	1.5	27	DF
7	spindle	low	4.4	15	DF
8	spindle	low	1.0	ND	died of lung cancer
9	spindle	low	2.3	60	died of disease
10	spindle	high	2.8	86	DF

ND: no data. DF: disease free

Immunohistochemical stain result

Case#	pan-cytokeratin	CK5/6	CK14	p63	EGFR
1	spindle cell (-), epithelial cell (+)	spindle cell (-), epithelial cell (+)	spindle cell (-), epithelial cell (+)	spindle cell (-), epithelial cell (+)	spindle cell (-), epithelial cell (+)
2	spindle cell (-), epithelial cell (+)	spindle cell (-), epithelial cell (+)	spindle cell (-), epithelial cell (+)	spindle cell (-), epithelial cell (+)	spindle cell (-), epithelial cell (+)
3	both cell types (+)	bothe cell types (+)	bothe cell types (+)	bothe cell types (+)	both cell types (+)
4	+	+	+	+	focal +
5	+	+	+	focal +	+
6	+	+	+	+	+
7	-	-	-	very focal +	+
8	+	very focal +	+	very focal +	+
9	very focal +	+	+	+	+
10	very focal +	-	very focal +	-	+

Conclusions: Even though the basal-myoepithelial markers showed expression in most of the spindle cell carcinomas, no single marker was consistently present. Also,

the staining can be very focal in its expression within a given tumor. Caution should be applied in excluding spindle cell carcinoma purely based on negative stains, especially for core biopsies. Morphologically low grade tumors can demonstrate an aggressive clinical course. Therefore, the pathological grade of spindle cell carcinoma has limited value in predicting clinical outcome.

208 Novel Biomarker To Aid in Differentiating Infiltrating Ductal Versus Infiltrating Lobular Carcinoma of the Breast
BVS Kallakury, GM Sheehan, CG Broustas, CE Sheehan, UN Kasid, JS Ross. Georgetown University Hospital, Washington, DC; Albany Medical College, Albany, NY.
Background: Given the well-documented differences in the development, progression and management of invasive ductal (IDC) and lobular (ILC) breast carcinomas, identification of novel biomarkers to aid in their histologic distinction is essential. Loss of E-cadherin expression has been reported in a majority of ILCs, and thus many practices have added it to their immunohistochemical panel as a reliable marker for distinguishing invasive breast carcinoma. Recent studies by this group, revealed that BRCC2 is expressed in virtually all cases of ILC. The purpose of this study was to evaluate the differential expression of BRCC2 and E-cadherin, an established marker of differentiation between IDC and ILC, in the same set of randomly selected group of breast cancers.
Design: Formalin-fixed, paraffin-embedded tissue sections from 110 cases of invasive mammary carcinoma (73 ductal carcinomas (IDC) and 37 lobular carcinomas (ILC) were immunostained by automated methods (Ventana Medical Systems Inc., Tucson, AZ) using custom generated BRCC2 and commercially available E-cadherin (DAKO, Carpinteria, CA) antibodies. For each protein, tumor immunoreactivity was semiquantitatively scored based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%) in all cases.
Results: Diffuse cytoplasmic BRCC2 positivity was noted in 37/37 (100%) ILC as compared to 58/73 (79%) IDC (p= 0.003). Loss of E-cadherin membranous immunoreactivity was noted in 26/31 (84%) ILC as compared to 20/55 (36%) IDC (p<0.0001). For the diagnosis of ILC, the sensitivity of BRCC2 expression was 100% and the specificity was 21%. For loss of ECAD staining, the sensitivity was 84% and specificity was 64%. For the BRCC2+/ECAD- phenotype, seen in 91% of ILCs, the sensitivity for ILC was 93% and the specificity was 60%.
Conclusions: BRCC2 expression is seen in 100% of ILC. When BRCC2 expression is combined with ECAD expression loss, there is a small loss (7%) of sensitivity for the diagnosis of ILC with a substantial gain in specificity (39%). Thus, BRCC2 immunostaining can complement ECAD staining loss in the classification of lobular versus ductal phenotype and may be of future use in the prognosis assessment and selection of therapy for invasive breast carcinomas.

209 Significance of Radial Scar Diagnosis in Breast Core Biopsy and Correlation with Follow up Surgical Excision
A Kanbour-Shakir, YC Teh, M Bonaventura, A Soran. Magee-Womens Hospital of UPMC Health System, Pittsburgh, PA.
Background: Radial scar (RS) is a benign breast lesion composed of fibrous central core surrounded by radiating ducts/lobules with varying degree of epithelial hyperplasia and adenosis. Current literature suggests that the presence of RS on core biopsy of a mammographic suspicious lesion are often associated with invasive/noninvasive breast cancer on excisional biopsy. Also women with radial scars had a risk of breast cancer almost twice that of women without the scars. This is a retrospective review of RS found in breast core biopsy specimens, associated histopathologic changes and the correlation with the histopathologic changes of the follow up surgical excision.
Design: Computer search of all breast core biopsies over the past five years (2003-2007) at Magee-Womens Hospital with the diagnosis of radial scar were evaluated. The histopathologic changes including the presence of associated cancer (invasive carcinoma and in situ) and/or high risk lesions (HRL): atypical ductal hyperplasia {ADH}; lobular carcinoma in situ {LCIS}/atypical lobular hyperplasia {ALH}; and papillomas; associated microcalcifications; and benign lesions (fibroadenoma {FA}, sclerosing adenosis {SA}, ductal hyperplasia {DH}, columnar cell changes {CCC}, or pseudoangiomatous stromal hyperplasia {PASH}), were tabulated and correlated with those of the follow-up surgical excision.
Results: A total of 195 out of 13,424 (1.45%) breast core biopsy specimens during 5 years period had RS diagnosis. Of those 153 (78.5%) had associated microcalcifications. 90/195 (46%) had RS and associated cancer and/or HRL.

RS associated pathologic findings in 195 core biopsies

Total	Benign	Cancer (invasive/in situ)	ADH	LCIS/ALH	Papilloma
Core biopsies (195)	105	18	30	11	31

These findings mandated surgical excision. Of the remaining 105, 72 (68.6%) had associated microcalcifications. Only 53/105 (50.5%) had follow up surgical excision with the following pathologic findings: 19 (36%) had cancer and/or HRL (6 cancers, 9 ADH and 4 LCIS/ALH). The other 34 had benign lesions.
Conclusions: 46% of breast core biopsies with RS had associated cancer or high risk lesions (HRL) mandating surgical excision. 36% of breast core biopsies with RS and benign lesions had cancer or HRL on follow-up surgical excision. The high incidence of atypia and malignancy identified in this group of patients justify the surgical removal of all RS cases diagnosed on core biopsy.

210 Best Practice Guidelines for the Pathological Evaluation of Reduction Mammoplasty Specimens – A Surgical Conundrum
R Kanthan, JL Senger, R Chibbar. University of Saskatchewan, Saskatoon, Saskatchewan, Canada.
Background: Surgical specimens of breast reduction mammoplasties generates a laboratory report from the histopathological examination of a few adhoc random sections.This practice was critically reevaluated to establish best practice guidelines for the handling of such specimens.
Design: A ten year (1996-2007) computer based data search for reduction mammoplasty specimens at the Saskatoon Health Region was undertaken. The cases were reviewed for the following parameters: patients age, number of tissue sections submitted, average weight of the breast specimen and the pathological findings.
Results: The ten year search identified 1334 cases (30-140 cases/year). 65.8% of the 1320 female patients ranged from 20-40 years of age; 40-60 were 30.3% and 3.9% were >60 years of age. In contrast 71.4% of the 14 males were under the age of 30. The weight of a single female breast specimen, right or left, ranged from less than 100g (4.35%) to more than 1 Kg (11.65%), while the average weight of male breast tissue removed ranged from 30-400g with less than 100g in 42.9%. In the female breast specimens (94.53%) the average number of blocks submitted per breast were 3.19 (1-29) with 2 sections taken in 46.8% and 4 sections in 40.5%. The average number of blocks submitted per male breast was 2.071 with 2 sections taken in 42.9% and four sections in 35.7%. 65% of the female breast cases had no pathological abnormalities. Pathological changes in 467 cases included fibrocystic change, apocrine metaplasia (19.4%), fibrosis (1.27%), fibroadenomatoid, fibroadenoma (1.05%), ductal hyperplasia-usual/typical (0.3%), sclerosing adenosis (0.37%), DCIS (0.07%), atypical hyperplasia (0.1%) and microcalcification (0.15%). In the male breast specimens 28.6% had no abnormalities. Pathological findings included: gynaecomastia (57.1%), fibrocystic change, apocrine metaplasia and epithelial hyperplasia in 6.1%. More than one pathology was seen in 7% (males) and 11% (females).
Conclusions: Our findings recommend continued pathological evaluation of reduction mammoplasty specimens in males and females. We recommend 3 section sampling for younger patients and 5 section evaluation in older patients (40 years or older) as best practice guidelines. Additional sections are recommended in identified breast cancer risk cases. Further understanding of epithelial/stromal changes in breast carcinogenesis may necessitate increased breast sections in younger breasts as additional tools for predictive breast cancer risk assessment in the future.

211 Prevalance and Significance of Perineural Invasion (PNI) in Invasive Breast Carcinoma
SG Karak, A Ricci, Jr. Hartford Hospital, Hartford, CT.
Background: Lymphovascular invasion (LVI) is a poor prognostic feature in breast cancer. Perineural invasion (PNI), a sign of aggressive behavior potential in other tumor systems is less frequently observed in mammary carcinoma, and hence has been less well studied. The present work was conducted to determine the frequency of PNI in mammary carcinoma and to describe the relationships between PNI, tumor characteristics (including LVI) and clinical outcome.
Design: The Hartford Hospital pathology database was reviewed for cases of invasive mammary carcinoma diagnosed from 2000 to 2002. The search was then narrowed to include only those cases reporting PNI, LVI, or both. These targeted reports were then reviewed to abstract clinico-pathologic data with regard to patient age, tumor stage and nuclear/histologic grade. Histologic review was performed on all PNI(+) cases. Comparisons between tumor characteristics associated with PNI and LVI were generated. Nodal status and patient outcome were obtained from cancer registry records.
Results: From a total of 1136 cases of invasive mammary carcinoma diagnosed from 2000-2002, 13(1.14%) and 146(12.9%) showed PNI and LVI, respectively. Of the 13 patients with PNI, 5/13 (38.5 %) also had LVI. Both PNI and LVI were associated with higher T-stage and intermediate to high NG/HG (see table).

Cases with LVI (N=146) age range 31-95; mean 57.3

T1a	1	HG-I	5	NG-I	1
T1b	7	HG-II	51	NG-II	38
T1c	46	HG-III	79	NG-III	104
T2	61	Unk	11	Unk	3
T3	6				
T4	0				
Tx	25				

Cases with PNI (N=13) age range 46-87; mean 63.6

T1a	0	HG-I	0	NG-I	0
T1b	1	HG-II	8	NG-II	7
T1c	1	HG-III	2	NG-III	5
T2	8	Unk	3	Unk	1
T3	1				
T4	0				
Tx	2				

After a mean follow up of 5.9 years, only 1/13 patients was DOD; 2/13 were DOC; 10/13 were alive at last contact (8/13 A w/o D and 2/13 with unknown tumor status).
Conclusions: PNI is a relatively rare histologic feature in invasive breast carcinoma occurring ten times less frequently than LVI. Tumor characteristics associated with PNI include higher T-stage, higher tumor grade and LVI. Despite this, patients with PNI can expect a meaningful survival at 5 years with appropriately aggressive adjuvant therapy (only one of 13 patients in this study was known DOD after mean follow up of 5.9 yr). When observed in tissue sections PNI should be reported (for completeness) but its role as a poor prognostic feature remains questionable.

212   Correlation of the Gene Expression Profile of Primary Breast Carcinoma with Clinical Outcome
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Background: Optimal treatments for breast cancer rely on classic clinicopathologic parameters, which are not specific enough to identify all subgroups at high risk of treatment failure. DNA microarray technology allows analysis of the RNA expression of several thousand genes simultaneously. Discovery of new clinically useful prognostic subgroups, currently not found by conventional parameters, is made possible by use of gene expression profiling. Our aim was to investigate the global molecular profile of invasive primary breast cancer with 15 year follow up to discover prognostically important genes associated with survival and potential new biomarkers for therapeutic targets.
Design: RNA (100ng) extracted from 104 tumors and 17 normal breast samples was analyzed using Affymetrix whole genome microarrays. Following normalization using dCHIP, statistical filters were used to identify significant differentially-expressed genes between tumor and normal; lymph node positive vs node negative tumors; patients who relapsed or died within 5 years vs survivors. Principal component analysis was performed on resulting genelists; literature mining software was used to identify pathways affected; individual mRNAs were extensively investigated for associations with clinicopathologic characteristics. qRT-PCR was used to validate results.
Results: 7448 transcripts were differentially expressed (P=0.0068) between tumor and normal. 998 transcripts (P=0.0009) and 1369 (P=0.0013) between tumors associated with relapse or death within 5 years vs those that did not. 12 mRNA associated with unfavorable factors and 24 with favorable prognostic import were identified. Patent office searches showed that this panel of 36 transcripts had not previously been associated with cancer and many do not appear to be represented on earlier microarrays where the information is publicly available. Amongst these transcripts, multivariate Cox regression analysis of validating qRT-PCR results showed PRAME to be independently unfavorable in terms of overall survival (OS) (P=0.02) and relapse free survival (RFS) (P=0.026); SNIP to be independently unfavorable for OS (P=0.005); and TMEM25 to be independent in terms of both OS (P=0.001) and RFS (P=0.011).
Conclusions: We have identified a panel of 36 gene transcripts independently associated with favorable or unfavorable outcome in primary breast carcinomas by multivariate analysis. These results identify new prognostic subgroups that may provide new potential therapeutic targets.

213   Urokinase-Type Plasminogen Activator (uPA) Is a Marker of Microinvasion in High Grade Ductal Carcinoma In Situ Identified in Core Biopsy
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Background: Ductal carcinoma in situ (DCIS) with microinvasion may be biologically different from DCIS without associated invasion. In order to establish potential differences we compared the imunohistochemical (IHC) expression of biomarkers in two groups, DCIS with microinvasion and DCIS without invasion.
Design: Archival core biopsies (CB) performed from 01/2006 - 04/2008 were reviewed, 19 high grade DCIS cases associated with microinvasion (M+) and a control group of 19 DCIS cases without invasion in CB or subsequent excision (M-) were identified. Cases were matched for the nuclear grade of DCIS. Clinicopathologic data were reviewed and IHC evaluation of ER, PR, HER-2, p53, Ki67, COX2, p16, Cyclin D1, uPA, uPAR and uPAI-1 was performed. Two tailed Chi square test analysis was used for statistical analysis.
Results: M+ group was upstaged to T1 in 36% of subsequent surgical excisions, while all M- cases remained Tis. M+ patients were slightly younger than the M- patients (mean age = 56.0 vs. 61.9), had similar radiologic presentation (mass vs. suspicious calcifications) and a similar frequency of birads 4C (7/19 in M+ vs. 6/19 in M-). The family history of breast cancer was more common in M- group (7/19 vs. 2/19, *p*: 0.056) while the personal history of breast carcinoma was only found in M+ group in 37% (86% of them contralateral). Morphologic features, including nodule formation, necrosis, periductal fibrosis and lymphocytic reaction were similar in both groups. uPA expression in DCIS cells was significantly higher in M+ group (95% M+ vs. 67% M-, *p* = 0.029). Although Ki-67 showed a trend for higher values in M+ group (M+ mean=27% vs. M- mean=15%), the frequency of high proliferation index (Ki67 positive in >10% nuclei) was not significantly different. The IHC results of the remaining biomarkers showed no differences between the two groups.
Conclusions: DCIS with microinvasion shows a trend for higher proliferation index and has increased uPA expression as compared to DCIS with no associated invasion. The utility of uPA as a prospective marker to identify DCIS with increased risk for progression to invasive disease should be confirmed in outcome studies.

214   Invasive Lobular Carcinomas Do Not Express Basal Cytokeratin Markers CK5/6, CK14 and CK17
N Khilko, J Wang, DG Hicks, P Tang. University of Rochester Medical Center, Rochester, NY; RTI Health Solutioon, Research Triangle Park, NC.
Background: Basal subtype of ductal carcinomas been shown to be associated with high grade tumors and poor clinical outcome. Pervious study has shown that basal markers CK5/6 can be detected in up to 17% of invasive lobular carcinomas (ILC). Here we studied the expression of three basal cytokeratin markers (CK5/6, CK14, and CM17) in ILC.
Design: Fifty-three ILC were identified from the files of Department of Pathology. Clinical and pathological information including patients' age, tumor size, multifocality, ER, PR and HER2 status, lymphovascular invasion, perineural invasion, and status of lymph nodes were reviewed and recorded. One representative section from each case was also stained with antibodies to basal markers CK5/6, CK14 and CK17, and luminal

markers CK8 and CK18. Any strong cytoplasmic stain was considered as positive.
Results: Among the 53 cases of ILC, 42 were classic lobular carcinomas, 6 were tubular-lobular carcinoma, and 5 were pleomorphic lobular carcinomas. There was no significant difference among these three groups in patients' age, tumor size, uni- or multifocality, expression of ER and PR, lymphovascular invasion, perineural invasion and lymph node metastasis. The only statistically different factor was HER2 over-expression, which was only observed in pleomorphic ILC (p=0.0073). None of the 53 cases was positive for any of the three basal cytokeratin markers. 51/53 cases expressed luminal cytokeratin markers CK8 and CK18, and the two negative cases were both ER and PR positive classical ILC.
Conclusions: All three basal CK markers failed to show expression in any of ILC in the current study, suggesting that very few cases of ILC will demonstrate a basal phenotype. More studies are needed to further investigate molecular classification in lobular lesions.

215   Delayed Formalin Fixation Effect on Breast Biomarkers
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Background: Delayed formalin fixation (DFF) may invalidate hormone-receptor and HER2 analyses. Invalid results of tumor markers could significantly impact the type of adjuvant therapy a patient receives and potentially impact outcome. The purpose of this study was to determine the effects of progressive DFF on breast biomarkers.
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° (ON). Two tissue microarray blocks were constructed. ER, PR, HER2 immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) were performed. Q-score was used to evaluate ER and PR. For HER2 FISH and IHC, the ASCO-CAP (2007) recommendations were followed. HER2 FISH was considered invalid whe at least one of the following was present, vague cellular outline, non-uniform signal, and/or poor nuclear resolution in more than 75% of the tumor cells. Statistical analyses including non-parametric sign test and exact McNemar's test were used.
Results: All ten cases were invasive ductal carcinomas. Q-score ≥6 was identified in 5 cases for ER and 4 for PR. Mean Q-score started to decline at 2 hr mark for ER and 1 hr mark for PR. Lowest score was at 8 hr mark for ER and ON for PR. HER2 FISH started to be invalid for interpretation at 2 hr mark (p < 0.008).
Conclusions: Delayed formalin fixation more than 1 hr has significant effect on HER2 FISH. Although changes due to DFF were not statistically significant for ER and PR, it is recommended not to delay fixation for more than 1 hr as well and not to store specimens ON.

Results of biomarkers changes with time.

	ER*	PR*	HER2 FISH	
	Mean Q score (range)	Mean Q score (range)	# valid cases	p-value
0 min	7 (7-7)	6.75(6-7)	10	NS
10 min	7 (7-7)	6.75(6-7)	10	NS
30 min	7 (7-7)	6.75(6-7)	10	NS
1 hr	7 (7-7)	6 (4-7)	10	<0.008**
2 hr	6.8 (6-7)	6 (4-7)	2	<0.008
4 hr	6.4 (6-7)	6 (4-7)	2	<0.008
8 hr	5.6 (3-7)	5.75 (4-7)	2	<0.008
ON	5.8 (5-7)	5.25 (3-7)	1	<0.004

*p-value for ER and PR was calculated using sign test (not significant). ** p-value for HER2 FISH was calculated using exact McNemar test. NS, not significant.

216   Analysis of Frozen Section Results of Sentinel Lymph Node in 896 Patients with Breast Cancer
A Kim, WJ Sung, YK Bae, SH Kang, SJ Lee. Yeungnam University College of Medicine, Daegu, Korea.
Background: Intraoperative frozen section (FS) of sentinel lymph nodes (SLNs) is widely used to determine whether axillary dissection should be followed in breast cancer patients. However there have been no standard guidelines for the number of sections, cutting interval and the use of immnunohistochemistry. We evaluated the usefulness and limitations of FS protocol which has been used in our institution for intraoperative SLN examination.
Design: We analyzed FS results of 896 patients who underwent intraoperative SLN biopsy between January 2005 and December 2007. Our institutional protocol is as follows. SLNs larger than 5mm were sliced in 2mm interval and all slices from one SLN were embedded in one block. Then two serial sections were cut for hematoxylin and eosin (HE) stain without trimming away the embedded tissue. The rest of the frozen tissue was formalin-fixed and paraffin-embedded for permanent section (PS). Two serial PSs from each block were taken, one for HE and the other for cytokeratin (AE1/AE3) immunostain. Cytokeratin stain was done in the cases with negative FS results. Metastatic SLNs were graded according to the AJCC cancer staging manual (6th edition) as isolated tumor cells (ITCs), micrometastasis (>0.2 to 2mm) and macrometastasis (>2mm). The results of FS and PS were compared with regard to the pathologic diagnosis and the standard was based on the results of PS.
Results: Among 896 patients, 810 patients (90.4%) had invasive carcinoma and 86 (9.6%) patients had ductal carcinoma in situ. The average number of SLNs was 3 per patients. Total 240 (26.8%) patients were found to have metastatic SLN(s) in the final pathology report. In 34 patients, the PS diagnoses were negative but the PS diagnoses were positive (false negative rate, 3.8%). Another 10 (1.1%) cases which were negative on FS had ITCs on the cytokeratin immunostains. The rate of micrometastasis in the false negative cases was 85.3%. False negative results were caused by interpretation error (29.4%) or technical problems (no tumor cells visible on FS, but tumor cells visible on PS) (70.6%).

Conclusions: The false negative rate of our protocol for FS of SLN was very low. The failure of FS was largely caused by the failure to detect micrometastasis. Therefore, FS is a reliable method for intraoperative SLN examination if a very stringent protocol is used.

217 The Role of Adipocytes in Mammary Carcinoma Microenvironment

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Background: Recent studies suggest a role for tumor microenvironment in mammary carcinogenesis. The most numerous cell population surrounding breast cancers, adipocytes are surprisingly the least studied cell in this microenvironment. This study examines the expression of a cell cycle regulator, p53, in adipocytes surrounding various ductal proliferative lesions with or without invasive carcinoma.

Design: 293 cases of ductal intraepithelial neoplasia (DIN) were retrieved from the surgical pathology files of our institution. These included 119 DIN associated with invasive carcinoma: and 174 pure DIN. A representative slide from each case was evaluated for immunoexpression of p53 in the adipose tissue. Results were interpreted as follows; negative (0-10%) and positive (>10%). The expression of p53 was correlated with patients' age and type of proliferation.

Results: The p53 expression increased with increasing patient age with the highest expression among patients aged 81 to 96 years (44%), followed by those 51-80 (29%), 41-50 (25%) and 21-40 (17.5%) years of age. The most extensive adipocyte p53 expression was in cases associated with invasive carcinomas (36%) compared to 15% for those without invasive carcinoma. The p53 expression in the adipocytes did not directly correlate with the grade of DIN.

Conclusions: Adipocytes in breast have multiple functions including elaboration of cell cycle regulators that may influence mammary carcinogenesis. The findings in our study of 1) an increase in adipocyte p53 expression with increasing patient age in parallel to the increasing frequency of breast carcinoma with age and 2) the most extensive p53 expression in association with invasive carcinomas support such a role.

218 Expression of COX-2, p16, and p53 in Ductal Intraepithelial Neoplasia

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Background: Breast cancer is a heterogeneous disease with multiple genetic pathways influencing tumor growth and progression. Identification of markers overexpressed during carcinogensis can have diagnostic and therapeutic implications. Recent studies have implicated COX-2 expression as an early marker in breast carcinogenesis, while p16 overexpression, an inhibitor of cyclin-dependent kinases 4 and 6, has been noted in a variety of cancers including hormone dependent tissues. p53 tumor suppressor gene plays an important role in the regulation of the apoptotic response of cells following exposure to stress. To determine the role of these alterations in mammary carcinogenesis, a series of breast lesions ranging from low-risk ductal intraductal neoplasia (L-R DIN, intraductal hyperplasia) to invasive carcinoma were evaluated with immunostains for COX-2, p16 and p53.

Design: Eighty cases were retrieved from the surgical pathology files of our institution. These included 20 L-R DIN, 25 flat DIN 1 (flat epithelial atypia), 13 DIN 1, ≤ 2 mm (atypical ductal hyperplasia), 10 DIN 1, > 2 mm (DCIS, grade 1), 32 DIN 2 (DCIS, grade 2), and 17 DIN 3 (DCIS, grade 3). Thirty cases co-existed with invasive carcinoma. Immunohistochemical stains for COX-2, p16, and p53 were performed according to manufacturer's guidelines. Immunostains were graded based on intensity and percentage of positive cells and were reviewed by 3 pathologists.

Results: COX-2 had maximum expression in normal mammary tissue, L-R DIN and flat DIN 1. The maximum p16 expression was noted in DIN 3 and DIN 1, ≤ 2mm. p53 had a gradual increase in expression from normal mammary epithelium to DIN3. The results are summarized in table 1.

Cox-2, p16 and p53 expression in DIN lesions

Marker	Normal, N=71	L-R DIN, N=20	Flat DIN1, N=20	DIN1, ≤2mm, N=13	DIN1 (DCIS, grade 1), N=10	DIN2 (DCIS, grade 2), N=36	DIN3 (DCIS, grade 3), N=17
COX-2	79%	81%	74%	39%	50%	61%	53%
p16	70%	69%	65%	100%	89%	84%	100%
p53	27%	50%	52%	70%	50%	77%	94%

Table 1

Conclusions: Our data suggests an inverse relationship between COX-2 expression on one hand and p16 and p53 expression on the other hand in a range of breast lesions, with a higher rate of COX-2 over-expression in early lesions and increased p16 and p53 expression in more advanced lesions. Particularly notable was the heterogeneity of expression among cases of a specific subtype as well within a given case.

219 Basal-Like Breast Cancer Displays Distinct Promoter Methylation Profiles

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Background: Recent microarray profiling studies on breast cancer have identified distinct subtypes that are associated with different clinical outcomes. Promoter methylation of several known or putative tumor suppressor genes occurs frequently during the pathogenesis of breast cancer. We propose that distinct subtypes of breast cancer are likely to contain distinct promoter methylation profiles.

Design: A panel of 10 gene promoters was assessed by quantitative multiplex methylation-specific PCR in 114 invasive ductal carcinomas representing the three major subtypes (57 luminal, 24 HER2, and 33 basal-like) based on immunohistochemical findings of estrogen receptor, progesterone receptor, HER2, CK5/6, and epidermal growth factor receptor.

Results: Significant differences in methylation levels among the three subtypes of breast cancer were found for APC (p < 0.05), BRCA1 (p < 0.01), CDH1 (p < 0.05), HIN1 (p < 0.01), RASSF1A (p < 0.01), TWIST (p < 0.01), and cumulative methylation index (CMI) (p < 0.05). APC, HIN1, RASSF1A, and TWIST methylation levels and CMI were significantly lower in basal-like subtype compared to luminal or HER2 subtype, whereas BRCA1 methylation levels were significantly higher in basal-like subtype compared to luminal and HER2 subtypes. CDH1 methylation levels were significantly higher in HER2 subtype compared to luminal subtype.

Conclusions: Basal-like breast cancer displays differential methylation profiles compared to luminal and HER2 subtypes. Detection of methylation levels for some genes is potentially useful as epigenetic markers in breast cancer classification.

220 Expression of Estrogen Receptor β and BRCA1 in Male and Female Breast Cancer

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Background: There has been recent interest in the role of estrogen receptor (ER)β expression in breast carcinomas. A previous report showed frequent ERβ expression in male breast carcinomas (MBC). Data comparing ERβ expression in MBC and female breast carcinomas (FBC) is limited. ERβ expression has also been shown in "triple negative" (ERα-, PR-, HER2-), BRCA1-mutation-associated FBC. In this study, we compared expression of BRCA1, ERβ, ERα, progesterone receptor (PR), androgen receptor (AR) and HER2 in MBC and FBC, and examined the possible relationship between ERβ and BRCA1.

Design: Immunohistochemical stains for BRCA1, ERβ, ERα, PR, AR, 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). Absence of nuclear staining for BRCA1 suggested presence of a BRCA1 mutation. A positive reaction for the steroid receptor stains was defined as nuclear staining present in at least 10% of tumor cells. HER2 was scored according to CAP guidelines. HER2 FISH results were obtained in cases with equivocal and positive HER2 staining.

Results:

	MBC	FBC	p-value
BRCA1 (-)	0/28 (0%)	2/77 (2.6%)	1
ERβ	24/25 (96%)	64/66 (97%)	1
ERα	23/27 (85.2%)	49/73 (67.1%)	0.085
PR	14/27 (51.9%)	40/76 (63.9%)	1
AR	20/27 (74.1%)	46/72 (63.9%)	0.473
HER2	6/26 (23.1%)	14/63 (22.2%)	1

No BRCA1 mutations were identified in the MBC cases. Both triple-negative MBC were ERβ-positive. BRCA1 staining was absent in two of 12 triple-negative FBC. ERβ staining was present in both cases. The ERβ-negative MBC and one of two ERβ-negative FBC were positive for ERα.

Conclusions: In our population, the expression of all studied markers, including ERβ, was similar in MBC and FBC. The expression of ERβ in triple-negative tumors with absent BRCA1 staining in FBC is confirmed. BRCA1 mutation was not noted in any MBC, possibly due to the small sample size.

221 Detection of Minimal Residual Disease in Blood and Bone Marrow in Early Breast Cancer

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Background: The significance of circulating tumor cells (CTCs) in blood and disseminated tumor cells (DTCs) in bone marrow (BM) in early stage breast cancer is currently not entirely known. In this study, we investigated the occurrence of CTCs and DTCs in patient's with early stage breast cancer and evaluated the correlation of their presence with other standard prognostic markers.

Design: We collected peripheral blood before surgery and BM aspirates (from bilateral iliac crests) at the time of primary breast surgery. CTCs were detected by immunomagnetic's (Cell Search, Veridex, LLC) using 3 tubes, each containing 7.5 ml of blood. Presence of one or more CTCs in either tube was considered as positive. The BM aspirates were subjected to Ficoll Hypaque density gradient separation and 10 cytospins which were immunstained using pancytokeratin antibody (cocktail of AE1/AE3, CAM5.2, MNF116,CK8,CK18). The presence of any cytokeratin(CK) positive cell/cells with morphological features consistent with tumor cells was regarded as positive for DTC. The presence of CTCs in blood and DTCs in BM was correlated with standard prognostic markers including tumor stage (T1 vs T2), lymph node status, estrogen, progesterone receptor, and HER2 status of the primary tumor.

Results: The study included 92 patients belonging to stage T1(49) and T2 (43). CTCs were detected in 25 of 81 (31%) and DTCs in 18 of 67 (27%) patients. CTCs and DTCs were nearly equally prevalent in the lymph node(LN) positive and LN negative groups; DTCs in 8 of 31 LN + vs 10 of 36 LN- (P=0.856): CTCs in 10 of 25 LN+ vs 15 of 46 LN- group (p=0.657). Similarly, CTCs were detected in 12 of 43 stage T1 vs 12 of 46 stage T2, in 18/54 ER + vs 7/27 ER-, in 14/45 PR+ vs 11/37 PR-, in 1/4 HER2+ vs 24/77 HER2 – patients respectively. DTCs were detected in 11/36 stage T1 vs 6/29 stage T2, in 11/48 ER+ vs 7/19 ER-, in 8/36 PR+ vs 10/31 PR-, 0/4 HER2+ vs 18/63 HER- patients respectively. There was no significant correlation between the occurrence of CTCs in blood and DTCs in BM with tumor stage (T1 vsT2), ER, PR and HER2 positivity.

Conclusions: 1).CTCs and DTCs in blood and bone marrow occurred in 31% and 27% of patients with T1 and T2 breast cancer.2)The presence of minimal residual disease in blood and BM did not correlate with other prognostic indicators including lymph node positivity, tumor stage, ER, PR status or HER2 overexpression.3)The lack of correlation

with lymph node positivity indicates independent modes of dissemination of the tumor cells to these homing sites.

222 Detection of Disseminated Tumor Cells in Bone Marrow of Patients with Breast Cancer: Comparison of Two Enrichment Techniques
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Background: Presence of disseminated tumor cells (DTCs) in bone marrow (BM) of patients with breast cancer is associated with a poor prognosis. Currently, there is no standardization of the methods for the detection of DTCs in BM. We sought to compare the performance of two enrichment techniques for detecting DTCs in BM, including density gradient separation(DGS) and immunomagnetic separation(IMS) using EpCam beads for detection of DTCs.
Design: BM (10 ml) was aspirated from bilateral iliac crests in EDTA primed tubes at the time of primary breast surgery. One half of the specimen was layered with lymphoprep, centrifuged at 1,500 rpm for 10 minutes, the buffy coat was separated, washed in PBS, centrifuged and from the cell pellet, 10 cytospin smears were made; one of which was stained by Papanicalaou method and the others(9) immunostained with pancytokeratin antibody. The other half of the BM aspirate were enriched with affinity columns containing EpCAM (epithelial marker) magnetic beads (Miltenyi Biotech). The purified cell populations were pelleted onto two slides, one of which was stained with Pap method and the other immunostained with pancylokeratin antibody. The overall sensitivity of both methods for detecting CTCs was calculated.
Results: We included 40 BM specimens for a direct comparison of DGS and IMS. Pap stained cytospin smears of DGS separated cells showed abundant hematopoietic cells (1000) without any recognizable tumor cells in any of the cases. Rare isolated CK positive cells, morphologically consistent with tumor cells were detected in 9 of the 40 cases (22.5%). IMS yielded cells ranging from 5-50 in each slide. Pap stained cytospin smears showed few cells (3-10) in 3 of the 40 cases (7.5%) with morphological features, consistent with tumor cells. CK immunostaining of the destained Pap stained smears confirmed the presence of CK positive cells in these three cases. The overall sensitivity of the DGS was higher than IMS 22.5% vs7.5% in this study. The difference in sensitivity between the two techniques reached statistical significance (p=.009, Fishers exact test).
Conclusions: 1. The sensitivity of DGS was significantly higher than IMS for isolation of DTCs in BM.2. IMS yields more DTCs, which can allow further phenotypic characterization beyond identification in some if not all cases.3. The decreased sensitivity of IMS is most likely due to loss of cells in the process of enrichment and expression of EpCAM in some but not all DTCs.

223 Immunohistochemical Correlation of p53 with Unamplified Chromosome 17 Polysomy in Invasive Breast Carcinoma
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Background: We have peviously demonstrated that unamplified chromosome 17 polysomy in invasive breast carcinoma (IBC) is associated with several increased adverse prognostic indicators in contrast to patients with neither amplification or polysomy (Mod Pathol 21(Supp 1):177: 41A, 2008). p53 IHC has been found to correlate with polysomy 17 in bladder carcinoma and head and neck squamous cell carcinomas. While p53 expression has been demonstrated to correlate with some adverse prognostic factors in IBC, its expression has not been correlated with HER2 amplification status or with polysomy 17. Herein, we examined p53 expression by IHC in cases of IBC showing unamplified polysomy 17 and compared it to cases with HER2 amplification and those with neither amplification or polysomy.
Design: A total of 135 cases of IBC, divided in three groups: **N** (neither polysomy or amplification, 44 cases), **P** (polysomy 17 without HER2 amplification, 53 cases) and **A** (HER2 amplification without polysomy 17, 38 cases) were compared for p53 immunostaining. The percentage of positively stained cells and the intensity of staining on a 1 to 3 scale were noted. p53 results were also correlated with HER2 and CEP17 copy numbers.
Results: 30/135 (22.2%) cases were positive for p53. All positive cases had > 25% cells with 2-3+ score with 74% cases having > 75% cells with 3+ score. 1+ staining in <10% cells was considered negative. 6.8% (3/44) of **N**, 24.5% (13/53) of P and 36.8% (14/38) of the **A** group were p53 positive. There was a statistically significant difference between the N group and both the P and A groups (p= 0.018 and 0.0008 respectively; 2 proportion Z test). The difference between the P and A groups was not statistically significant (p= 0.20). The mean CEP17 copy numbers for p53 negative and p53 positive cases was significantly different [**2.6** (95% C.I 2.4-2.7) and **3.1**(95% C.I 2.7-3.4), p= 0.007]. The mean HER2 copy numbers for p53 negative and p53 positive cases was also significantly different [**5.2** (95% C.I 4.3-6.1) and **10.8** (95% C.I. 7.4- 14.1), p=0.000].
Conclusions: There is increased expression of p53 in unamplified polysomy 17 similar to that seen in HER2 amplification. P53 positivity correlates with CEP17 and HER-2 copy numbers. Therefore, p53 positivity, is additional support for the adverse prognostic significance of unamplified polysomy 17.

224 Utility of Frozen Section for Intra-Operative Evaluation of Sentinel Lymph Nodes in Breast Cancer
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Background: Intraoperative evaluation of axillary sentinel lymph nodes (SLN) allows the surgeon to complete axillary dissection in one setting at the time of primary breast surgery if diagnosed as positive for metastatic tumor. There is currently no consensus regarding the optimal method for intra-operative evaluation of SLNs. This study was initiated to evaluate the utility of frozen section (FS) for intraoperative evaluation of SLNs in patients with and without pre-operative neoadjuvant chemotherapy and their ability to detect macro and micrometastasis.
Design: SLN biopsies performed in 2007 with FS analysis for immediate assessment were included. SLNs were sliced at 2 mm intervals along the short axis and submitted entirely for FS which were stained by hematoxylin and eosin method. Final pathologic examination (FP) of formalin fixed and paraffin embedded tissue was regarded as the gold standard. FP included one H&E stain of the first section and cytokeratin immunostain of the third section. The overall sensitivity and specificity of FS for detecting metastatic tumor in patients with and without chemotherapy for detection of micro(>0.2mm and<2mm) and macro metastasis(>2mm) was calculated.
Results: The study included 358 patients; 73 received neoadjuvant chemotherapy (NC). FP examination revealed metastatic carcinoma in 11/73 (15.1%) patients who received NC and in 82/285 (28.8%) patients who did not receive NC (non-NC). The average size of micro-metastasis was 0.75 mm and the average size of macro-metastasis was 5.4 mm. In the NC group, FS detected 5/11 patients with metastasis to SLN; 2 with micro-metastasis and 3 with macro-metastasis. In the non-NC group, FS detected 50/82 patients with metastasis; 15 with micro-metastasis and 35 with macro-metastasis. The sensitivity of FS for detecting micro-metastasis was 33% in the NC group compared to 43% in the non-NC group. The sensitivity of FS for detecting macro-metastasis was 60% in the NC group compared to 76 % in the non-NC group. The specificity was 100% for both groups. The false negative rate in the NC group vs non-NC group was 66.7% vs 60% for micrometastasis and 40% vs 23.9% for macro-metastasis.
Conclusions: 1) The overall sensitivity of FS for intraoperative evaluation for detection of micrometastasis (33%vs43%) and macrometastases (60%vs76%) was lower in patients who received pre-operative chemotherapy in comparison to those who did not receive any such therapy. 2)The false negative rate for detection of macro-metastasis was lower than that of micro-metastasis in both groups.

225 Expression of the Stem Cell Markers ALDH1 and EZH2 in Triple Negative Invasive Breast Carcinomas
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Background: Markers associated with ER, PR and HER2/neu negative invasive breast carcinomas (triple negative, TN) may help in the diagnosis and treatment of these aggressive tumors. Emerging studies indicate that stem cells may be responsible for tumor initiation. There is no literature on expression of stem cell markers in TN carcinomas. We characterized ALDH1 and EZH2 expression in a cohort of uniformly treated TN patients and evaluated the ability of EZH2 to predict a TN phenotype.
Design: A tissue microaary was constructed with 52 invasive TN carcinomas. ALDH1 and EZH2 expression was evaluated using conventional immunohistochemistry (IHC) and AQUA analysis. By IHC, EZH2 was scored as 1 (negative), 2 (weak), 3 (moderate) and 4 (strong). Scores 1 and 2 were considered low, and scores 3 and 4 were considered high. ALDH1 was assessed as positive or negative in the epithelium and stroma, following published criteria. EZH2 expression was compared between TN and a cohort of non-TN tumors.
Results: EZH2 expression was nuclear. High EZH2 was strongly associated with a TN phenotype (p<0.0001) compared to all other non-TN tumors. High EZH2 expression was noted in 42 of 52 (81%) TN tumors and was significantly associated with high histological grade (p=0.0002). ALDH1 expression was cytoplasmic. Twelve of 52 (23%) TN tumors were positive for ALDH1 in the epithelial component while 32 (61%) showed ALDH1 in the stromal fibroblasts, which has not been previously reported. ALDH1 expression was not associated with tumor grade, size, angiolymphatic invasion or lymph node metastasis in TN carcinomas. However, when combined with high EZH2, both markers showed a very strong association with histological grade (p=0.0006) and tended to have larger size (p=0.06). We did not find a good correlation between expression of these markers by IHC and AQUA analysis.
Conclusions: High EZH2 expression predicts a TN phenotype in breast cancer, and identifies poorly differentiated tumors with high histological grade. ALDH1 is more commonly expressed by stromal cells than epithelial cells in TN carcinomas. Concordant high EZH2 and positive epithelial ALDH1 expression is associated with high histological grade and larger size in TN breast cancers. There is poor correlation between expression of these markers between immunohistochemistry and AQUA analysis, which warrants further investigation.

226 ER/PR Positive and ER/PR Negative DCIS: Morphologic Characterization in a Cohort of Age-Matched Cases
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Background: Histologic and molecular data support the concept that DCIS is a heterogeneous disease comprised of different lesions with different biological behavior, in part driven by their hormonal receptor expression. We systematically assessed morphology and associated atypia in cohorts of age-matched ER/PR+ and ER/PR- DCIS.
Design: ER/PR- and age-matched ER/PR + DCIS cases diagnosed between 01/07 and 06/08 were retrieved. We evaluated core and excisional biopsies and recorded the following parameters: nuclear grade, size, architecture, basaloid features, presence of necrosis, calcifications, inflammation, desmoplasia, and associated intra-epithelial lesions including flat epithelial atypia (FEA), atypical ductal hyperplasia (ADH) and lobular carcinoma in-situ (LCIS). We also recorded upgrade to invasive carcinoma on excision.
Results: Twenty ER/PR- and 23 age-matched ER/PR+ DCIS were compared. The mean size of both group was comparable (ER/PR+ DCIS: 1.0 cm, 0.2-3 cm, ER/PR- DCIS: 0.9 cm, 0.2-2.5 cm). The vast majority of ER/PR- DCIS (90%, 18/20) were high nuclear grade vs. 17% (4/23) of ER/PR + tumors. More than one nuclear grade was noted in ER/PR + DCIS only (13%, 3/23). Necrosis and desmoplasia were more common in ER/

PR- DCIS (75% vs. 35%, and 75% vs. 26%, respectively). Basaloid histological features were rare, seen in 10% ER/PR- DCIS only. ER/PR+ DCIS had a variety of histological patterns (solid 10/23, cribriform 8/23 or micropapillary 5/23), whereas ER/PR- DCIS was mainly solid and/or apocrine (11/20) or cribriform (5/20). ADH, FEA and/or LCIS was noted in 70% (16/20) of ER/PR+ DCIS, being significantly less common in ER/PR- DCIS (20%, 4/20). FEA was only associated with ER/PR+ DCIS. Core biopsies were performed in 11/23 and 14/20 of ER/PR+ and ER/PR- cases respectively. In 45% (5/11) ER/PR+DCIS, the core biopsy was diagnosed as ADH with or without FEA. Upgrading to invasive carcinoma was only seen in ER/PR- DCIS (10%, 2/20 cases).
Conclusions: ER/PR- DCIS has fairly uniform morphology, and is commonly of high nuclear grade. Basaloid features, though rare, are noted in ER/PR- DCIS only. ER/PR+ DCIS is morphologically more heterogeneous with different architectural patterns, and areas of different nuclear grades. ADH and LCIS are significantly more common in ER/PR+ DCIS. FEA was present in ER/PR+ cases only. The presence of ADH and/or FEA on core biopsies was associated exclusively with ER/PR+ DCIS on excision. When matched for age at diagnosis, the size of ER/PR+ and ER/PR- DCIS is similar, but upgrading to invasive carcinoma is higher in the ER/PR- group.

227 Aberrant Expression, and Potency as Cancer Markers/Targets, of Splicing Regulators in Breast Cancer
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Background: SR " splicing regulator " and hnRNP " heterogeneous nuclear ribonucleoprotein " proteins are mRNA binding proteins that regulate several aspects of mRNA metabolism, from splicing/polyadenylation processes to translational control. Since ample evidences exist to demonstrate a connection between alternative splicing and cancer, we have examined the expression of several of these regulators in breast cancer.
Design: Four SR (9G8, SC35, SRp20, ASF/SF2) and 1 hnRNP (hnRNP A1) proteins were analyzed using tissue microarrays of 277 invasive breast carcinomas. Immunostainings were interpreted using a score combining the percentage of stained cells and the intensity of staining. Staining localization, clinical and histopathological data were recorded for each case.
Results: The study population showed the following characteristics: 9-year median follow-up, median age 54 years, median tumor size 18mm, invasive ductal carcinoma (77.3%), histological grade divided in grade I (12.3%), grade II (39%) and grade III (45.8%), node negative disease in 56.7%, ER+ tumor in 65%, PR+ tumor in 58.4%, and 12% of HER2 over-expressing tumors. Two of the 5 proteins studied, hnRNP A1 and SC35, were statistically correlated to survival. A high hnRNP A1 expression was correlated to poor outcome when considering relapse-free survival (p=0.05), distant metastasis-free survival (p=0.03) and overall survival (p=0.02). A low SC35 expression was associated to poor prognosis when considering relapse-free survival in the overall population (p=0.04), but also in the node negative subgroup (p=0.04). Combination of a high hnRNP A1 expression and a low SC35 expression was strongly correlated to poor prognosis (relapse-free survival, p=0.01 in the overall population and p=0.006 in the node negative subgroup). In addition, the hnRNP A1 subcellular localization was highly correlated to prognosis, with identification of a subset of poor prognosis patients showing strong hnRNP A1 cytoplasmic staining.
Conclusions: Aberrant expression of key-proteins regulating post-transcriptional control of gene expression is thus correlated to clinical outcome in breast cancer. Such key-factors, participating to the protein diversity, may provide new prognostic or therapeutic target possibilities.

228 Breast Carcinoma of Combined Histologic Grade 2: Prognostic Significance of Immunohistochemical Features
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Background: When breast cancer (BC) is graded by the Nottingham system, an important proportion of the cases are classified into the category of grade 2 (G2), irrespective of the immunophenotype. In order to determine some discriminating features with prognostic significance, we analyzed the expression of Bcl2, Estrogen and Progesterone receptors (ER/PgR), Ki67, Her2 and p53 in a series of BC of G2.
Design: A total of 811 cases of invasive BC were studied from our database. Median clinical follow-up was 78 months (range 15 to 313 months). According to the Nottingham system, 292 cases (36%) were G2. Ages ranged from 20 to 86 years (median 57 years). Tumor size ranged from 2 to 110 mm (median 19 mm). Immunohistochemical (IHC) staining was performed for Bcl2 (cut-of 50%), ER (cut-of 10%), PgR (cut-off 10%), Ki67 (cut-off 15%), p53 (cut-off 20%) and Her2 (2+ and <30% 3+ confirmed by FISH). Tumors were classified according to the immunophenotype as luminal "low-risk" (Ki67/p53 <20%), luminal "high-risk" (Ki67/p53 >20%), Her2-positive and triple-negative (ER/PR/Her2-negative). Significant associations were identified using Chi-square and Fisher's exact test. Actuarial survival was calculated by the Kaplan-Meier method (log rank test). Multivariate analysis was determined by Cox's proportional hazard model. A p-value <0.05 was considered significant.
Results: Tumors were predominantly of ductal type (93 %), with <20 mm in size (56%) and negative lymph nodes (66%). Bcl2 was high in 67% cases, ER-positive in 84%, PgR-positive in 76%, low Ki67 in 64%, p53-negative in 86% and Her2-positive in 15%. Based on IHC findings, 64% tumors were classified as luminal "low risk", 14% as luminal "high risk", 15% HER2-positive and 7% as triple negative. Survival analysis showed a significant correlation with axillary lymph node status (p=0.01), Bcl2 (p=0.01), Ki67 (p=0.04) and immunophenotype (p=0.04). However, there was no association with tumor size, ER/PgR or p53 (all p>0.05). Moreover, a multivariate analysis showed that the lymph node status was the only significant independent predictor of survival (p=0.023).

Conclusions: In our series of BC of G2, the results show that the stratification of the tumors according to their immunophenotype and the levels of Bcl2 and Ki67 have clinical relevance for those patients. However, the axillary lymph node status is the only independent prognostic factor.

229 Acidophilic Nuclear Inclusions Are Fairly Specific for Florid Duct Hyperplasia among Proliferative Breast Lesions
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Background: It is believed that breast usual ductal hyperplasia (UDH) represents a different lineage of cells than atypical ductal hyperplasia (ADH) and low-grade ductal carcinoma in-situ (LG-DCIS). Many of the cytologic features distinguishing UDH from ADH/DCIS have been well established. In 1991, Tavassoli et al noted "helioid" nuclear inclusions (Ultrastructural Pathol., 15(3):267) in "hyperplasias"; however, to the best of our knowledge there has not been any systematic study on the incidence and specificity of these inclusions.
Design: 89 cases from were selected including 40 cases of UDH, 15 cases of UDH, and 34 cases of LG-DCIS. The cases were reviewed by three pathologists who developed a consensus diagnosis for the lesion, and the presence or absence of intranulcear inclusions was documented.
Results: Two distinct types of nuclear inclusions were discerned. The first, termed white nuclear inclusions (WNI), consisted of white areas of chromatin clearing associated with an ill-defined vacuolated appearance, as well as other artifactual nuclear changes in the background, and when present tended to be widespread. The second, termed acidophilic nuclear inclusions (ANI), exhibited round, dense, sharply demarcated eosinophilic globules with occasional targetoid condensation and well defined borders. ANI were identified in 50% of cases of UDH, while none were observed in any cases of ADH or LG-DCIS. WNI were seen in 47.5% of UDH cases, but were not specific as they were also present in cases of ADH (40%) and LG-DCIS (23.5%). No inclusions of any kind were seen in 20% of cases of UDH, 60% of ADH, and 73.5% of LG-DCIS. Overall, the presence of acidophillic inclusions was significantly associated with a diagnosis of UDH compared to ADH (p=0.019) and LG-DCIS (p=0.008).
Conclusions: Acidophilic nuclear inclusions are a common, specific feature found in usual ductal hyperplasia of the breast, and may be helpful in distinguishing UDH from some cases of ADH and low grade DCIS. The significance of these inclusions is unknown, but may be related to the "heliod inclusions" seen by Tavassoli et al., or to the BROCNs (biotin-rich optically clear nuclei) seen in morular-type proliferations in women, which are now thought to be surrogate products of beta-catenin pathway alterations (Hum Pathol 2004, 35:869). Elucidating the nature of these inclusions may provide insight into the pathogenesis of usual ductal hyperplasia of the breast.

230 Detection of Occult Metastases in Sentinel Lymph Nodes: Comparison of a Limited Widely Spaced and a Comprehensive Narrowly Spaced Paraffin Block Sectioning Strategy
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Background: The NSABP B-32 protocol is examining whether patients with initially negative sentinel lymph nodes (SLNs) who have occult metastases detected on deeper levels and cytokeratin (CK) immunohistochemical stains are at risk for regional or distant metastases. For all initially negative SLNs, the experimental B-32 sectioning protocol examines H+E and CK stains approximately 0.5 and 1.0 mm deeper into the paraffin blocks (2 levels; wide spacing) and was designed to detect virtually all metastases larger than 1.0 mm; smaller metastases may be missed. This pilot quality assurance study compares detection using the experimental B-32 protocol to a more comprehensive sectioning protocol designed to detect virtually all metastases larger than 0.2 mm (multilevel; narrow spacing).
Design: All SLNs were sectioned grossly at close to 2.0 mm and all sections embedded in paraffin blocks. For clinical treatment, a single H+E section was examined from each block. For 50 cases with 1-4 SLNs and all SLNs negative, additional sections were evaluated every 0.18 mm through the block until no tissue remained. All sections were stained with CK and sections at 0.5mm and 0.9mm were also stained with H+E.
Results: 7 of 42 (16.7%) cases harbored occult metastases; 5 cases (11.9%) were detected with the B-32 protocol and 2 additional cases (4.8%) were detected on sections that would not have been evaluated (p=0.25; correlated proportions). The largest metastasis in each case was 0.03, 0.04, 0.15, 0.5, and 0.6 mm for the B-32 strategy and 0.15 and 0.3 mm for the two cases detected only by the more comprehensive strategy. Median number of levels examined per block on the comprehensive protocol was 11 (range 1-20); the B-32 protocol was fixed at 2 levels (median 2; range 1-2). Median thickness of node sections in the block was 2.1 mm (range 0.3-3.7 mm).
Conclusions: The B-32 protocol with two widely spaced levels detected 5 of the 7 cases with occult metastases identified. To detect the two additional cases, 11 narrowly spaced levels completely through the paraffin block were required. Although more comprehensive sectioning of SLNs detects additional micrometastases, the data suggest diminishing returns and reduced cost effectiveness for the comprehensive strategy. The limited number of occult metastasis positive cases limits statistical power; however, large scale studies comparing these two strategies are prohibitively expensive.

231 Metaplastic Sarcomatoid Carcinoma of the Breast: Poor Overall Survival Is Independent of the Amount of Sarcomatoid Component
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Background: Metaplastic carcinomas of the breast with a pure or predominant sarcomatoid morphology are known to have a poor clinical outcome, but it is unclear whether metaplastic carcinomas with smaller sarcomatoid components are equally aggressive.

Design: We examined the clinicopathologic features of forty-eight cases of metaplastic sarcomatoid carcinoma with the sarcomatoid component comprising from 5% to 100% of the tumor. Those with pure sarcomatoid morphology were included if they had immunohistochemical expression of cytokeratin and/or associated ductal carcinoma in situ and if the patients did not receive neoadjuvant chemotherapy. Patients with low-grade fibromatosis-like metaplastic tumors and other metaplastic tumors with a low-grade spindle cell component were excluded.

Results: Axillary lymph node dissection or limited axillary node excision was performed in 31 patients. Lymph node involvement occurred in 10 patients. Seven of these had a carcinomatous component comprising 75% to 90% of the primary tumor. Two patients with a carcinomatous component comprising 15% and <5% of the primary tumor had only micrometastases, and one patient without an overt carcinomatous component had only isolated tumor cells in a lymph node. Clinical follow-up was available for 38 patients and ranged from 2 to 201 mos (median 25 mos). Ten patients (26%) had locoregional recurrence, sixteen patients (42%) developed distant metastases, and eight (21%) had both. Locoregional recurrence-free survival, distant metastasis-free survival and overall survival were not dependent on the amount of sarcomatoid component in the primary tumor. Five-year overall survival for patients with ≥95% and <95% sarcomatoid component in the primary tumor was 46% ± 11% and 44% ± 14%, respectively (P=0.50). Five-year overall survival for patients with >10% and ≤10% sarcomatoid component in the primary tumor was 45% ± 9% and 40% ± 22%, respectively (P=0.50).

Conclusions: Metaplastic carcinomas of the breast with an intermediate to high-grade sarcomatoid component are aggressive tumors. Lymph node metastases tend to occur in patients without a predominant sarcomatoid component, but the amount of sarcomatoid component is not significantly associated with disease recurrence or overall survival.

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232 Epithelial-Mesenchymal Transition Cells Are Enriched after Hormonal Therapy
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Background: Epithelial-mesenchymal transition (EMT) is a multi-step process in which cells reorganize cytoskeleton, lose cell-cell junctions, and acquire mesenchymal spindle-shaped morphology. EMT is thought to promote the progession and invasion of cancer cells. The role of EMT in cancer progression and metastasis has been specifically demonstrated in breast cancer. A set of EMT gene signatures has been identified including up-regulation of vimentin, MMP2/3 and down-regulation of E-cadherin. Our unpublished data and other studies suggest that the EMT pathway may be involved in breast cancer stem cell development and proliferation. We have previously shown that breast cancer stem cells are resistant to chemotherapy and hormonal therapy, and that the stem cell population is increased after these treatments. Our aim in this study was to evaluate whether EMT cells are also enriched after conventional treatment.

Design: Twenty-three (23) paired ER positive paraffin embedded breast cancer samples before and 3 months after letrozole treatment were stained with vimentin and E-cadherin using an immunohistochemical (IHC) method. Positivity was evaluated using the Allred scoring scheme. Immunofluorescence co-staining using vimentin and pan-cytokeratin in biopsy samples was used to confirm the co-expression of vimentin and pan-cytokeratin in tumor cells. We also measured the MMP2 RNA level, using Q-RTPCR, in samples from 60 patients before, 10-14 days and 3 months after letrozle treatment.

Results: IHC demonstrated significant enrichment of vimentin expressing tumor cells after letrozole treament (P<0.05). Immunofluorescence studies confirmed the co-expression of pan-cytokeratin and vimentin in tumor cells. The MMP2 RNA level was significantly increased at 10-14 days and 3 months after letrozole treament (P<0.0001 and P<0.00001, respectively) compared with pre-treatement samples. However, we did not find a decrease of E-cadherin expression in post letrozole treatment samples using IHC, which may be due to the low baseline level of E-cadherin in the tumor cells.

Conclusions: The association of EMT with treatment resistance has been inferred by several articles. We and others have shown the EMT pathway may be involved in breast cancer stem cell development and proliferation. We herein provide the first direct clinical evidence that EMT cells are enriched following conventional therapy and the EMT pathways may be associated with treatment resistance.

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233 Extranodal Extension in Metastatic Axillary Lymph Nodes among Breast Cancer Patients
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Background: The number of positive lymph nodes (LN) is the only node-related prognostic factor for breast cancers recognized by the AJCC. Additional node-related factors have been evaluated by many groups. Our current pilot study aimed at characterizing extranodal extension with the associated tumor features, while our future studies will focus on the clinicopathologic correlation of extranodal extension to serve as a surrogate for survival.

Design: We analyzed data from patients diagnosed with invasive breast cancer between years 1998-2000. 292 patients were divided into 3 groups: 1) Patients with negative LNs (NoMet) group (56%), 2) Patients with positive LNs but without extranodal extension (MetNoExtraN) group (14%), 3) Patients with positive LNs and extranodal extension (MetExtraN) group (30%).

Results: A large percentage (68%) of patients with metastatic breast cancer to the LNs showed extranodal extension. Our studies found the number of positive LNs to be significantly higher in MetExtraN than in MetNoExtraN (Table 1), while the total number of LNs examined are not different between these 2 groups. Also, the maximum dimension of the positive LNs is larger in MetExtraN.

Table 1: Mean numbers and size of axillary lymph nodes

	# Positive LNs	# Total LNs	Max Dimension (cm)
MetNoExtraN	2***	15	1.3**
MetExtraN	7***	17	2.1**

***p<0.0001, **p=0.006, Mann-Whitney

The mean tumor size of NoMet group was 1.9 cm, smaller than that of MetNoExtraN (3.1) and MetExtraN (3.3), but there was no significant difference between the latter two groups (Mann-Whitney). Comparison of Nottingham grades showed statistical difference only between NoMet and MetExtraN group (Table 2).

Table 2: Age and percentage of Nottingham grades

	Mean Age	Grade 1	Grade 2	Grade 3	Total
NoMet***	61	9%	60%	31%	100%
MetNoExtraN	54	3%	56%	42%	100%
MetExtraN***	57	1%	44%	54%	100%

***p= 0.0009, Chi-square

Conclusions: Our data suggest that although the total number of examined LNs, the Nottingham grades, and the tumor size were the same in breast cancers with and without extranodal extension, the MetExtraN group distinguishes itself with more positive LNs and larger tumor volume in the metastatic lymph node. The finding of extranodal extension may play an important role in patient's treatment and prognosis, which will be further investigated in our studies.

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234 A New Approach to Breast Carcinoma Detection: NYBR-1 Teamed up with Mammaglobin and Gross Cystic Disease Fluid Proteins
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Background: NY-BR-1 is a novel differentiation marker with mRNA expression restricted to breast, testis, prostate and breast cancer. It has been shown by Immunohistochemistry (IHC) that NY-BR-1 was present solely in ductal epithelium of normal breast tissue. Invasive carcinoma of the breast and carcinoma in situ were positive for NY-BR-1, whereas most other tumors and normal tissues are negative. The exceptions are that one-third of sweat gland carcinomas and two percent of prostate carcinoma are positive for NY-BR-1. In a study by Varga et al (2006) forty-nine percent of lymph node metastasis were shown to be positive. Mammaglobin and GCDFP-15 are known metastatic breast carcinoma markers.

Design: IHC was performed using NY-BR-1, Mammaglobin and GCDFP-15 antibodies on different tissue arrays. NY-BR-1 was initially evaluated by IHC on arrays containing breast cancer, other cancers and normal tisssues. All three markers were further evaluated on metastatic multi-tumor arrays and metastatic breast carcinoma to node arrays. The arrays were scored based the staining intensity and percentage of positive tumor cells: tissue cores which showed 1 (weak) in ≥5% of tumor cells and tissue cores which showed 2 (moderate to strong) or above in ≥1% tumor cells were counted as positive.

Results: Analysis of results in breast cancer with clinicopathologic variables in 68 cases found that NY-BR-1 positive rate is lower in groups with larger tumor size, higher histology grade and auxiliary node metastasis. In addition, (2/12) prostate cancers showed moderate staining, while all other non-breast carcinoma tumors were negative for NY-BR-1. In human breast cancer arrays containing 209 cores, 60.3%are NY-BR-1 positive, 35.7% are Mammaglobin positive and 36.2% are GCDFP-15 positive. The combined positive rate is higher (77.3%) than that of the individual markers. All three markers were negative on forty metastatic non-breast carcinomas. Twenty seven cases of metastatic breast carcinoma of the lymph node, lung and liver demonstrate that the combined positive rate of the three markers is 74%.

Conclusions: NYBR-1 in itself is a specific breast cancer marker demonstrating a higher rate of positivity compared to both GCDFP-15 and Mammaglobin. The combined positive rate of the three breast markers is higher than each individual marker in both primary breast carcinoma and metastatic breast carcinomas. The use of the three markers has the potential to be a valuable diagnostic tool for metastatic breast carcinomas.

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235 Influence of Cytokeratin 5/6, EGFR, p53 and Ki-67 Index on Pathologic Complete Response Rate to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancers: Preliminary Results from the I-SPY TRIAL (CALGB 150007/150012 and ACRIN 6657)
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Background: The I-SPY trial is a multi-institutional study of locally advanced breast cancers. The primary objective is to identify markers of response to neoadjuvant chemotherapy. Pathologic complete response (pCR) of triple-negative breast cancers (TNBCs) to neoadjuvant chemotherapy is associated with a relatively favorable prognosis. The aim of this study was to identify markers associated with pCR in TNBCs.

Design: Immunohistochemistry (IHC) for HER2, EGFR, p53, and Ki-67 was performed centrally on all core samples at baseline. ER and PR results were obtained from each institution. IHC for cytokeratin 5/6 (CK5/6) was performed on TNBCs. A comprehensive approach using microarray analysis, SSCP and sequencing was used to evaluate tumors for p53 mutations. Post-surgical specimens were reviewed centrally to determine residual cancer burden including pCR rate. P-values were calculated with Fisher's exact test.

Results: 221 patients have enrolled and completed therapy. Accrual of patients is ongoing. A total of 53 TNBCs were identified. TNBCs showed a significantly higher pCR rate, 40% (21/53), compared to ER+/PR+/HER2- tumors, 9% (9/101), p<0.0001. The table below shows pCR rate in TNBCs based upon cytokeratin 5/6, EGFR, Ki-67 and p53 status.

TNBC result	#pCR	Total	%	Lower 95% CI	Upper 95% CI	p-value
CK5/6+	8	19	42%	20%	67%	0.35
CK5/6-	7	26	27%	12%	48%	
EGFR+	4	10	40%	12%	74%	0.99
EGFR-	11	32	34%	19%	53%	
Ki-67<10%	1	6	17%	1%	64%	0.10
Ki-67≥10%,<25%	3	11	27%	6%	61%	
Ki-67 ≥25%	12	26	46%	27%	67%	
p53+ overexpression	8	30	27%	12%	46%	0.04
p53- overexpression	8	13	62%	32%	86%	
p53 mutant	12	33	36%	20%	55%	0.99
p53 wild-type	4	10	40%	12%	74%	

Conclusions: TNBCs showed a significantly higher pCR rate (40%) compared to ER+/PR+/HER2- tumors (9%). Lack of p53 overexpression in TNBC was associated with higher pCR rate as compared to p53-positive tumors. A similar association was not observed from the results of the p53 mutation analysis. Higher pCR rates were observed in TNBCs showing cytokeratin 5/6 expression and Ki-67 index >25%; however, these associations were not statistically significant. Tumor EGFR status was not associated with pCR rate.

236 Are FISH HER-2/neu Results the Same on Core Needle Biopsy and Excision in Patients with Breast Cancer?
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Background: HER-2/neu receptor amplification status is critical to the pathological work-up of and the guidance of therapy for breast carcinoma. Fluorescence in-situ hybridization (FISH) is the gold standard for HER-2/neu amplification evaluation, yet immunohistochemical staining (IHC) is a more widely used method due to its lower cost and easier accessibility. Multiple studies have shown FISH and IHC results to have high concordance rates. To our knowledge, a comparison between FISH results for core needle biopsy specimens (CNB) and FISH results for the subsequent excision specimens (EXC) has not yet been studied. We sought to determine the concordance rate of HER-2/neu FISH in CNB and EXC.
Design: We retrospectively evaluated the FISH and IHC results in both the breast CNB and the corresponding EXC of 125 patients with invasive carcinoma of the breast from 2002-2005. Standard breast biomarkers were performed on each specimen, including IHC for HER-2/neu receptor overexpression (0 to 3+, based on pre-ASCO 2007 evaluation criteria) as well as FISH for HER-2/neu amplification (scores of <2.0 interpreted as not amplified). The IHC were evaluated by a total of four pathologists. The FISH analysis was performed and interpreted by an independent cytogenetic lab.
Results: Comparison of FISH results of the 129 CNB to the 131 EXC of all 125 patients showed a FISH concordance rate of 92% (115/125).

FISH HER-2/neu Gene Amplification on CNB and EXC			
Concordant	115/125 (92%)	13 CNB+ EXC+	102 CNB- EXC-
Discordant	10/125 (8%)	6 CNB+ EXB-	4 CNB- EXC+

The IHC results of the same CNB and EXC showed a concordance rate of 98% (122/125). Comparison of the IHC results with the FISH results for all 260 cases examined showed 95% concordance (247/260).

IHC and FISH Results of 260 Cases (129 CNB and 131 EXC)			
IHC Score	# of 260 Cases (%)	% FISH Negative (#)	% FISH Positive (#)
0	52 (20%)	100% (52)	0% (0)
1	147 (57%)	93% (137)	7% (10)
2	40 (15%)	70% (28)	30% (12)
3	21 (8%)	14% (3)	86% (18)

Conclusions: The concordance rate of FISH and IHC (95%) in the population studied was similar to previously published results. Although the concordance rate of FISH between CNB and EXC is high (92%), it is not 100% concordant. In fact, IHC for HER-2/neu overexpression had a higher concordance rate (98%).

237 Expression of Sox9 Is Significantly Associated with Basal-Like Phenotype in Invasive Mammary Carcinoma
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Background: Basal-like tumors are a subset of breast cancer defined by molecular profiling that retain expression of basal keratins but lack estrogen and progesterone receptors and express low amounts of HER2. They have been found to be associated with a worse overall and disease-free survival. A handful of biomarkers for basal-like tumor have been identified, but the underlying factors that drive this phenotype are largely unknown. Sex-determining region Y-box 9 (Sox9) is an embryonic transcription factor that plays pivotal role in chondrogenesis and organogenesis of testis, pancreas, gastrointestinal tract, prostate gland, and skin. In this retrospective study we examined Sox9 expression in invasive ductal carcinoma (IDC) of the breast and correlated its expression with established prognostic factors.
Design: The study group comprised of 67 cases of poorly differentiated IDC retrieved from the files over a 5-year period (1997-2001). Tumor characteristics including size, lympho-vascular invasion, necrosis, lymph node metastasis, ER, PR, and HER-2 status were obtained from pathology reports. Immunohistochemistry was performed on formalin fixed, paraffin embedded tissue using a rabbit polyclonal antibody against Sox9. Data for cytokeratin 5/6 and beta4-integrin was available on these cases from our earlier studies. Nuclear staining of greater than 10% of tumor cells was considered positive for Sox9 expression. Statistical analysis was performed by Chi-square test using JMP 6.0 (SAS Institute).
Results: Of the total 67 poorly differentiated IDC cases, Sox9 expression was seen in 35 (52%). Of the 67 cases, 17 were ER/PR and HER-2 negative (triple negative)

with basal-like phenotype, 14 (82%) of these were Sox9 positive. Within this group of high grade IDC there was significantly increased Sox9 expression in basal-like breast cancer compared to the non-basal tumors (p = 0.0029). Moreover, Sox9 expression was also positively associated with two basal-like breast cancer markers, cytokeratin 5/6 (P<0.0001) and beta4-integrin (P<0.0001).
Conclusions: Sox9 is a novel biomarker for triple negative (basal-like) invasive mammary carcinoma; its expression is associated with a more aggressive phenotype and correlates with other basal-like markers. Further examining the roles of Sox9 in breast cancer and identifying its mammary gland specific targets may be useful to understanding the transcriptional regulation of basal-like phenotype.

238 Expression of CD44+/CD24- Cells in Breast Cancer
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Background: The cancer stem cells have been found recently to associate with tumor's development, drug resistance, and recurrence and have also become potential targets for cancer treatment. Two lineages of stem-like cell, side-population and CD44+/CD24-/low cells, have been identified in breast cancer cells lines, but there is little research on tumor tissue.
Design: Sixty-four cases of breast cancer tissues with invasive ductal carcinoma were examined with immunohistochemical stains for expression of CD44+/CD24- cells. The correlation between the CD44+/CD24- expression and other conventional biomarkers including ER, PR, HER-2, PS2, Bcl-2, and nm23 were analyzed. The association of the distribution and number of CD44+/CD24- cells with clinicopathological factors like tumour size, lymph node status and metastasis were explored. Expression of CD44 and/or CD24 was defined as over 1% of tumor cells positive for CD44 and/or CD24 staining while 5% was used as the cutoff for other markers.
Results: CD44+/CD24- cells were randomly distributed in breast cancer tissues. The expression of CD44+/CD24- cells were identified in 43 of 64 (67.2%) cases of the invasive ductal breast cancer, significantly higher than that of CD44-/CD24- *(p=0.001)* or CD44-/CD24+ *(p=0.007)* cells but similar to that of CD44+/CD24+ cells. The expression of CD44+/CD24- cells in breast cancers was neither correlated to that of other biomarkers of breast cancer nor to the clinicopathological factors examined.
Conclusions: CD44+/CD24-cells are randomly distributed in breast cancer tissue and not peripherally concentrated as predicted. The expression of CD44+/CD24- is significantly higher than that of CD44-/CD24- or CD44-/CD24+ in invasive ductal breast cancer. Similar expression of CD44+/CD24- and CD44+/CD24+ suggest the stem-like cells may be diversified in breast cancer. Expression of CD44+/CD24- was not associated with that of ER, PR, HER-2, PS2, Bcl-2, nm23 or with the clinical factors including patients' age, tumor size and lymph node metastasis.

239 Tumour Infiltration by CD8+ T Lymphocytes Is Associated with Patient Outcome in Breast Cancer
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Background: Tumour-related antigens can be recognised by cytotoxic T lymphocytes (CTLs) in the context of MHC class I-expressing tumours. Immunohistochemical identification of CD8+ T lymphocytes has been recently correlated to an improved overall survival in colorectal, ovarian, renal, and oesophageal carcinomas. The prognostic significance of CD8+ T-cells in breast cancer has been studied in relatively small numbers of patients and the available reports do not have comprehensive data about different localisation patterns and long-term follow-up.
Design: We have assessed the expression of tumour-infiltrating CD8+ T-cells using immunohistochemistry of tissue microarray preparations from a large cohort of 957 unselected breast cancer cases with long-term follow up and investigated the relationship with clinical outcome.
Results: CD8+ lymphocytes were identified in 510 cases (54%), 392 cases (41%) were positive for intratumoural CD8+ T-cells and 320 cases (33%) were positive for stromal CD8+ T-cells. Tumours were divided into 4 groups according to the expression and localisation of CD8+ cells; negative tumours (contained no CD8+ cells, n= 447), tumours contained only intratumoural CD8+ cells (defined as cases with CD8+ cells touching tumour cells, n= 190), tumours contained only stromal CD8+ cells (n= 118), and tumours contained CD8+ cells in both compartments (n= 202). Patients with only stromal CD8+ T-cell in their tumours showed a significant association with the best breast cancer specific survival (BCSS, *p*= 0.005) and the longest disease free interval (DFI, *p*= 0.001). This effect was retained in ER+ and HER-2+ tumour subgroups. In contrast, the subgroup with only intratumoural CD8+ cells showed the worst prognosis. In multivariate survival analysis including tumour size, stage, grade, and HER-2 status; stromal CD8+ cell expression was an independent predictor of patient outcome.
Conclusions: In conclusion, we have identified that degree and location of CD8+ T lymphoid cells in breast cancer tissue samples influences patient outcome in an independent manner. Further investigation of the functional activity of CD8+ lymphoid cell in breast cancer in general and in its various biological sub groups appears warranted.

240 Do Chromosome 17 Centromere Copy Numbers Predict Polysomy? A Fluorescence *In Situ* Hybridization and Microarray-Based CGH Analysis

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Background: Approximately 8% of breast cancers show increased numbers of chromosome 17 centromere(CEP17) by fluorescence *in situ* hybridisation(FISH; i.e. average CEP17>3.0 per nucleus). Currently, this pattern is considered to represent polysomy of chromosome 17. Chromosome 17 displays complex patterns of genetic aberrations, in particular involving its long arm, in *HER2* amplified cancers. We hypothesised that increases in copy number of CEP17 may not necessarily represent chromosome 17 polysomy and that these could result from gains of centromeric/pericentromeric regions of chromosome 17 or aneusomy of 17q with involvement of CEP17.

Design: Eighteen CEP17 polysomic cases, of which 7 displayed *HER2*:CEP17 ratios>2.2, and a control group of 10 CEP17 disomic cases. *HER2* gene status was determined using the LSI *HER2*/CEP17 dual-colour probe(Vysis) and analysed with the motorised Metafer Scanning System. Analysis of *HER2*/CEP17 probes was performed automatically by Metafer through the PathVysion V2 software. Microarray-based comparative genomic hybridisation (aCGH) was performed in all cases after microdissection to ensure >90% of purity of cancer cells. A 32K tiling path bacterial artificial chromosome microarray platform(resolution 50kb) was employed. Data were normalised and smoothed using adaptive weight smoothing(aws). Smoothed data were converted into categorical values: losses - <-0.12; gains - >0.12 and <0.45; amplification ≥0.45; no changes - ≥-0.12 and ≤0.12. Gains of the long or the short arm of chromosome 17 were defined as >50% of clones with aws ratios >0.12.

Results: A perfect concordance between FISH assay and aCGH analysis was found in assessing *HER2* gene amplification. Out of the 18 polysomic cases, only two were polysomic (ie displayed gains of both short and long arm of chromosome 17). 11 polysomic cases displayed gains of 17q with involvement of part of the centromere. The remaining five cases that did not harbour gains of 17q displayed amplification of the centromeric region.

Conclusions: Our results demonstrate that CEP17 copy numbers>3.0 do not reliably identify chromosome 17 polysomic cases and that gains of CEP17 copies are more often caused by aneusomy of 17q and/or amplification of CEP17.

241 Breast Carcinoma and Lobule Development in Males: 5 Year Review at the Ottawa Hospital

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Background: Breast carcinoma (BC) in men is rare, with <1% of BC found in men. Lobular carcinoma (LC) of breast in men is even rarer, reported mostly as single case reports. A few recent larger series showed pure LC ranges from 0.39% to 3.6%. However, none of these confirmed this by IHC. Although the rarity of male LC has been attributed to an absence of lobules in male breast, there is no recent data on the presence of lobules in normal adult male breast, from clinical gynecomastia or from adjacent to ductal carcinoma. Tavasoli found lobules adjacent to 2/3 cases of male LC. Thus, the frequency of lobules in male breast is of interest. These questions were addressed in a 5 year review of male breast surgical specimens.

Design: A computer search between January 2003 - February 2008 was done and all cases of male BC and gynecomastia were reviewed and divided in BC and non-malignant (NM) groups. The age, sub-type of cancer, stage, grade, and receptor status was also recorded. The presence of lobules was documented microscopically in available breast parenchyma. Using a lobular score (0=none, 1= poorly defined, 2= well developed), mean scores in the NM and BC groups were calculated and compared.

Results: Of 4853 BC, 16 were from males (.003%). We compared the mean age, staging and tumor grade with published data and found our sample to be representative. Two of the 16 (12.5%) were LC as defined by histology and IHC. Twelve out of 16 cases had uninvolved adjacent breast tissue. Three cases showed lobules; only one of which was an LC. In 106 NM breast cases, 9 cases had no breast parenchyma. The rest of 97 cases, microscopy showed no lobules in 48.5%, poorly defined lobules in 39.2% and well developed lobules in 12.4% of cases. There was a significant difference in mean lobular score in the NM and BC cases (p=.02).

Conclusions: Our results for BC are similar to those seen in the literature, except for a much higher % of LC, likely explained by the small sample size. In our study the development of lobules was not restricted to LC. Unexpectedly, the % of lobule formation in NM male breast tissue was not rare (well developed lobules in 12.4%) and ductal carcinoma. This increase of lobules in NM breast, does not support the contention that LC is rare in men because of lack of lobular development or that lobules are a risk factor for any type of cancer (let alone LC). Further study is necessary with respect to presence of lobules in truly normal male breast and their relation with altered hormonal milieu.

242 Evaluation of Lymphatic Involvement within Needle Biopsy for Breast Carcinoma. Is It Useful?

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Background: Lymph node status is still the most important prognostic factor for disease-free and overall survival in breast carcinoma patients. We evaluated lymphatic involvement in core needle biopsies (CNB) and compared the efficiency for positive lymph node detection, with results from frozen sections (FS), ultrasound (US) or computer tomography (CT) imaging, and fine needle aspirations (FNA).

Design: Six hundred and twelve patients were selected from among 1,106 surgical breast cases in our hospital from 2005 to 2008. Patients with neoadjuvant chemotherapy, ductal carcinoma in situ, benign lesions and recurrences were excluded. The CNB were performed by using 18 G to 11 G needles, and one to six cores were taken. The lymphatic involvement was evaluated by HE staining and occasionally by assistance from immunostaining of antibody D2-40. Lymph nodes were cut every two millimeter for FS. US guided FNA was performed for on any suspected lymph node. Imaging evaluation of lymph nodes using US and/or CT at screening was checked by electronic reports.

Results: One hundred and forty eight patients (24.2%) had positive lymph node(s) and 92 patients (62.2%) had only a single positive lymph node, including 8 patients with isolated tumor cells. Twenty six patients (18.8%) with positive lymph nodes (PLN), and 7 patients (1.5%) without PLN were found to have lymphatic involvement in CNB. Two cases with marked lymphatic involvement in CNB had PLN. PLN were seen in 15 patients (10.1%) by FNA before surgeries and PLN were found in 132 patients (89.2%) by FS. The false negative rate of sentinel lymph nodes in FS was 7.6%. FNA could detect PLN in 25.9 %, and imaging in 37.2%.

Conclusions: The efficiency for detection methods of PLN was best in FS, followed by imaging, FNA and CNB in that order. CNB was not an efficient predictor of PLN, but may be efficient to do negative lymph nodes. Marked lymphatic involvement in CNB should be documented in the reports.

243 The Effect of Withdrawal of Combined Hormone Replacement Therapy (HRT) on the Proliferation of Breast Cancers in Postmenopausal Women

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Background: Postmenopausal combined HRT has been shown to be associated with a slightly increased risk of breast carcinoma (BC). Recent registry data showed a decrease in the incidence of BC corresponding in time to the decreased used of HRT following the 2002 publication of the results of the Women's Health Initiative trial. Based on the proliferative effect of estrogen and progesterone on benign mammary epithelial cells, it was hypothesized that the observed drop in BC incidence may be due to decreased proliferation of BC following HRT withdrawal. However, apart from one small study, there is very limited information available regarding the effect of HRT on the proliferation of human BC in clinical material.

Design: We selected 404 invasive BC diagnosed in postmenopausal women with available core needle biopsy (CNB) and subsequent surgical excision for the study. Among these 312 patients never used HRT, while 92 patients were current users of combined HRT at the time of CNB (median duration of use: 10 years). Immunohistochemical assays for Ki67 were performed on 5μm thick paraffin sections. Slides were digitized using an Aperio ScanScope XT. The percentage of Ki67 positive tumor cells (proliferation index, PI) was determined in at least 10 separate tumor areas in corresponding CNB and excisions. Mean PI values were compared using the Wilcoxon signed rank test.

Results: All current users stopped HRT after diagnosis of BC on CNB, the median time between CNB and excision was 41 days. We found no significant difference between never and current users with regard to age, tumor size, histologic type, grade, presence of lymphatic invasion and nodal metastasis, and hormone receptor and HER2 status. The number of mitoses per 10 high power fields was significantly higher in excisional compared to CNB material in both current and never users. The PI of BC was significantly higher in never users compared to current users in both CNB and excision. We found no significant difference in PI determined in CNB and subsequent excison either in never or current HRT users.

Conclusions: Our results do not support the hypothesis that withdrawal of HRT results in decreased proliferation of BC contributing to the observed recent drop in BC incidence. These findings are in contrast to prior data obtained in benign mammary epithelial cells and likely reflect important differences in the regulation of cell proliferation in benign and malignant cells.

244 Conditional Deletion of the *LKB1* Gene in the Mouse Mammary Gland Induces Tumour Formation

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Background: Heterozygous germ-line mutations in the *LKB1* (*STK11*) gene cause Peutz-Jeghers syndrome (PJS), an autosomal dominant disorder characterised by hamartomatous polyposis of the gastrointestinal tract and an increased risk of colorectal, breast, and other cancers. To specifically assess the effect of loss-of-function mutations upon mammary tumourigenesis we generated a mouse model bearing homozygous mutations of *Lkb1* in the epithelial compartment of the mammary gland.

Design: We used gene targeting technology to generate a mouse strain in which the exons encoding the kinase domain of *Lkb1* were flanked by *loxP* sites. Crossing of these mice to a strain expressing Cre recombinase in the mammary gland alone resulted in *loxP* mediated deletion and a gene encoding only the N-terminal 155 amino acids of the full-length (436 amino acid) protein, deleting 152 amino acids of the protein, including the kinase domain. Tumourigenesis was monitored in female mice. Mammary gland tumours were formalin fixed and subjected to immunohistochemical analysis using antibodies against oestrogen and progesterone receptors, HER2, cytokeratin 14 and p63. RNA was extracted from five tumours, which were snap frozen, and subjected to microarray analysis using the Mouse Sentrix-6 V1.1 BeadChip (Illumina).

Results: Mammary gland tumours developed in these mice with a latency of 46-85 weeks and occurred in the thoracic or inguinal glands. These tumours displayed histological features similar to those described in breast cancers arising in patients with PJS and were characterised as grade two invasive ductal carcinomas or solid papillary carcinomas. At the genomic level, these tumours consistently displayed a luminal phenotype. Ingenuity Pathway analysis revealed significant activation of cell cycle,

cellular development and cancer networks, upregulation of cyclin D1 and activation of PI3K/AKT canonical pathway.
Conclusions: This mouse model of Lkb1 deficiency provides a potentially useful tool to investigate the role of *Lkb1* in tumourigenesis and to guide the development of therapeutic approaches.
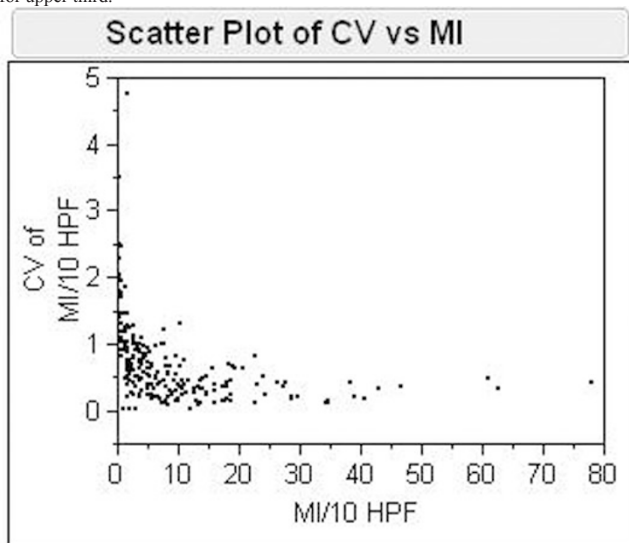
245 Mitotic Index of Breast Carcinoma: Understanding and Remedying Irreproducibility
JS Meyer, E Cosatto, HP Graf. St. Luke's Hospital, Chesterfield, MO; NEC Laboratories, Princeton, NJ.
Background: Histologic grading of invasive breast carcinoma has been practiced for more than 50 years. The Nottingham modification of the Bloom Richardson (NBR) system is endorsed by the College of American Pathologists and World Health Organization. While consistently prognostic, NBR grading has not been accepted as a standard on which therapeutic decisions can be based. Shortfalls in inter-observer reproducibility and inability to define a group of patients with sufficiently low probability of relapse (less than 10%) to justify omission of surgical adjuvant therapy are serious problems. Consensus favors the mitotic index (MI) as the most important of the three NBR variables.
Design: We undertook characterization of errors in measurement of the MI (mitotic count per 10 high power fields [HPF]) in a group of 328 specimens studied *en passant* in course of grading breast carcinomas for a tissue resource for researchers (N=214) and in clinical practice (N=114). MI was determined in two to six sets of 10 HPF, and standard error of mean and coefficient of variation (CV) were calculated for each specimen. Observed confidence limits and CVs were compared with theoretical values calculated by the binomial distribution model. Tertial cutoffs were determined.
Results: Mean MI of zero was observed in 40 specimens. In the other 288 specimens (Fig), observed CV means were 147% for lower third, 72.0% for mid third, and 34.6% for upper third.

Plots of observed and theoretical CVs for the 288 specimens were closely superimposed. Low and high tertial cutoffs for MI were 1.14 and 5.32.
Conclusions: Error in reproducibility of MI can be explained nearly entirely by sampling. The very high CVs of MI based on one set of 10 HPF is unacceptable. The NBR lower third cutoff of 7 mitotic figures per 10 HPF is far too high for our population, and could not be expected to separate good from poor prognosis. The CV can be reduced in proportion to the square root of the number of sets of HPF counted. At least 5 sets should be counted to establish that a low MI measurement is below a low tertial cutoff of 1 or 2 per 10 HPF for a given population. Counting multiple sets of HPF when the MI is low should increase the prognostic efficacy of the MI.

246 Objective Assessment of Lymphatic and Blood Vascular Invasion in Breast Carcinoma: Findings from a Large Case Series with Long Term Follow up
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Background: Vascular invasion (VI), encompassing both lymphovascular (LVI) and blood vascular invasion (BVI) plays an important role in breast cancer disease progression and influences patient prognosis. In a previous study we found that LVI is the predominant type of VI with minimal contribution of BVI and that use of immnohistochemical identification using podoplanin increase the accuracy of detection. The aims of this study were (1) to assess the frequency and the extent of LVI and BVI in a well characterized group of 1000 LN negative breast cancers with known 20 year follow up (2) to assess prognostic power of the VI in early breast cancer.
Design: Representative paraffin-embedded tumour sections were stained with the lymphatic marker podoplanin and the vascular markers CD34&CD31 to detect LVI and BVI respectively. Stained vessels were counted in each section and the results were correlated with clinicopathological criteria and patient survival.
Results: VI was detected in 218 (22% specimens); 211/218 (97%) were LVI while BVI was detected only in 7/218 (3%). The lowest frequency of LVI was in tubular

(5.6%) and mucinous (7.1%) carcinomas and was highest in invasive carcinoma of no special type (26%). LVIs were located in the peritumoural areas in 76% of the LVI-positive specimens; and were intratumoural in 24%. The frequency of involved lymphatic vessels ranged from 1 to 79. 167specimens (79%) had <5 LVIs, 23 (11%) had 6-10 LVIs and 22 (10%) had >10 LVIs. There was no significant difference between the frequency of LVI and development of recurrence or death from the disease. The presence of LVI was significantly associated with the development of metastasis, recurrence, worse disease free interval (DFI) and worse overall survival (OS). The 20-year OS rate was 85% in patients without LVI compared with 68% in patients with LVI. In multivariate analysis for OS and DFI; LVI retained significance (P <0.0001). LVI was able to stratify into different prognostic groups even in aggressive sub types such as basal-like breast cancer.
Conclusions: VI in breast cancer is predominantly of lymph vessels and is a powerful independent prognostic factor in lymph node negative breast cancer. The use of immunohistochemical staining with podoplanin increases accuracy of identification.

247 Angiosarcoma of the Breast: A Rare Complication of Radiation Therapy. Clinicopathologic Study of 33 Cases in a Single Institution
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Background: Angiosarcoma of the breast (BrAS) may involve mammary parenchyma or the skin of the breast. The latter is a rare but serious complication of radiotherapy (RT) following breast conservation management of breast carcinoma.
Design: We reviewed all cases of BrAS evaluated at our institution from 1987 to 2008. Only cases arising in the setting of RT for breast carcinoma were included in this study. Cases of primary mammary angiosarcoma were excluded. Tumors were graded using a three-tier system. Clinicopathologic data were obtained by medical records review. Survival was analyzed by Kaplan-Meier curve.
Results: The cohort included 33 women (mean age 66 yrs, range 34 to 87) who received RT for invasive ductal (n=27), lobular (1) or mucinous (2) breast carcinoma, or DCIS (3). Median interval between RT and BrAS was 78 mos (range 36 to 156). Lesions usually presented as violaceous nodules on the skin within the area of prior RT. Time from initial symptoms to diagnosis was a median of 2.9 mos (range 0 to 23). Morphologically, BrAS were composed of anastomosing vascular channels showing multilayering of endothelial cells and nuclear atypia. The majority of tumors (70%) were histologically high grade. All patients (pts) underwent surgery (85% mastectomy; 15% wide excision of chest wall). Eight (24%) pts received chemotherapy (7 neoadjuvant and 1 adjuvant). 42% of pts developed local recurrence and 9% developed distant metastasis (median 11 and 16 mos after RAS diagnosis, respectively). Recurrent disease was managed by surgery alone (n=4), chemotherapy (4), RT (1), surgery plus chemotherapy (4), or surgery, RT and chemotherapy (1). The median follow-up was 17.3 months. Twelve (36%) pts died. Median overall survival was 49 mos (5-year survival 39%).
Conclusions: BrAS is a rare complication of RT for conservative treatment of breast carcinoma. The latency period is approximately 80 months and the majority of angiosarcomas that develop subsequent to breast-conserving RT are morphologically high grade lesions. Local recurrences are quite common. Prognosis is similar to that seen in angiosarcomas of soft tissue and is poor with a median survival of 49 months and overall 5-year survival of 39%.

248 CXCL10 Tumor Expression in BRCA1 and Non-BRCA1-Associated Breast Cancer
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Background: CXC chemokines are important for enhancing immunity, regulating angiogenesis and mediating tumor metastases. One such chemokine, CXCL10 (Interferon-γ inducible protein 10) has been shown to inhibit tumor progression by recruitment of mononuclear cells and antagonizing the effects of angiogenic factors in the tumor microenvironment as well as promoting cancer progression by upregulating factors related to invasion and metastases. BRCA1-associated breast cancers tend to have a strong host lymphocytic response. Here we explore tumor cell expression of CXCL10 in a large cohort of pathologically well characterized BRCA1 and non-BRCA1-associated breast cancers.
Design: On TMAs constructed from tumors from 58 BRCA1 carriers, 64 BRCA2 carriers and 242 control patients from the Ontario Familial Breast Cancer Registry immunohistochemical analysis of CXCL10 and its receptor, CXCR3, was performed and scored using the Allred method. Tumor cell expression of both markers was compared with morphologic parameters and biomarkers including Ki-67, HER2, hormone receptors (HR), and basal cytokeratins (CK). The associations were analyzed by Chi-square or Fisher's exact test.
Results: CXCL10 tumor cell expression was found to be associated with margin circumscription (p=0.018), a peritumoral lymphocytic infiltrate (p=0.03), high grade (p=0.02) and a high Ki-67 index (p=0.0001). An association with lymphovascular invasion or lymph node metastases was not identified. Expression did not correlate with HER2, HR or basal CK expression. Rather than down-regulating expression of its receptor CXCL10 expression positively correlated with CXCR3 expression in tumor cells. Furthermore, in BRCA1-associated tumors, basal-like tumors were more likely to be positive for CXCL10 expression when compared with non-basal BRCA1-associated tumors (78% versus 22%), though this result did not reach statistical significance, likely due to the small sample size.
Conclusions: Our finding that tumor cell expression of CXCL10 is associated with a peritumoral lymphocytic infiltrate in addition to increased expression of its receptor, CXCR3, on tumor cells suggests that it may act in both a paracrine manner, affecting the tumor microenvironment, as well as in an autocrine manner, acting on the tumor

cells themselves. The effects of these circuits on breast cancer progression are complex and warrant further elucidation.

249 A Role for the AHR, an Environmental Pollutant Activated Receptor and Transcription Factor, in Mammary Tumor Progression

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Background: Polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCBs) likely play a role in human cancer initiation through activation of the AhR (aryl hydrocarbon receptor/transcription factor) and AhR-dependent induction of cytochrome P450 enzymes (CYP1A1 and CYP1B1). These enzymes metabolize at least some AhR ligands into carcinogenic intermediates, resulting in the genetic mutations that are the hallmark of cancer.Previous studies in our laboratory using murine and rat models of mammary tumorigenesis demonstrate nuclear AhR expression in primary mammary tumors, a result consistent with constitutive AhR transcriptional activity and suggestive of ongoing epigenetic AhR signaling.

Design: Here, we postulate that constitutively active AhR plays a role in regulating genes involved in tumor progression through epigenetic signaling, even in the absence of environmental ligands. To test this hypothesis, AhR activity in two human mammary tumor cell lines, Hs578T and BP1, was down-regulated by transient transfection or stable transduction of an AhR repressor (*AhRR*) or *AhR* siRNA, and the ability of these cells to exhibit an invasive phenotype were evaluated. Hs578T is a human malignant mammary carcinosarcoma and BP1 is a PAH-induced malignant mammary epithelial cell line, both of which exhibit invasive phenotypes *in vitro* and metastasis *in vivo*.

Results: AhR down-regulation in either line altered colony morphology in 3D cultures and significantly reduced tumor cell invasiveness in modified Boyden chambers were observed. Analyses of RNA arrays further suggested that the AhR contributes to tumor progression through transcriptional regulation of master gene regulators of tumor invasion.

Conclusions: These data agree with the hypothesis that constitutively active AhR promotes tumor invasion and that environmental AhR ligands may either enhance or alter this capability. Furthermore, they provide a specific molecular mechanism through which common environmental chemicals, exposure to which is associated with breast cancer incidence, may contribute to metastatic progression, the underlying cause of breast cancer deaths. Finally, these studies provide facile models with which to screen naturally occurring AhR inhibitors (e.g., bioflavonoids and polyphenols) as potential breast cancer preventatives or therapeutics.

250 Immunohistochemical Validation of Topoisomerase 2 alpha and BubR1 as Novel Breast Cancer Risk Genes in Benign Breast Tissue

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Background: In order to predict the risk of malignancy in histologically normal breast, we have developed a novel 32-gene malignancy-risk gene signature that was validated by real-time PCR. Here we report further validation of two of our top malignancy-risk genes, topoisomerase 2 alpha (TOP2A) and BubR1 at protein level.

Design: Using our novel malignancy-risk gene signature, we stratified 18 histologically normal breast (HNB) tissues as "high-risk" normals (HRNs, n=9) or low-risk normals (LRNs, n=9). All HRNs and LRNs were from peri-menopausal female patients (mean ages: 52 and 51 years respectively)**.** All archival sections from mastectomies of these patients were reviewed to select blocks with maximum numbers of histologically-normal terminal duct lobule units (TDLUs). Control set: Six invasive ductal carcinomas (IDC). 5μ thick serial sections were stained for TOP2A and BubR1 proteins, using standard immunohistochemical (IHC) protocols. TOP2A staining index (% of stained cells) and BubR1 H-scores based on the product of positively stained cells (%) and immunointensity (0/1+/2+/3+) were all specimen types (TDLUs present in the "HRN" and "LRN" samples and the selected IDCs).

Results: TOP2A immunstain was nuclear; BubR1 was cytoplasmic. The immunoreactivity in the tumor cells and in the benign acinar and ductal epithelium of all of the TDLUs was scored. There was significantly higher immunoreactivity for TOP2A (t. test, P < 0.01) and BUBR1 (t. test, P < 0.001) in histologically normal breast tissues, which had been predicted to be at high risk of malignancy using our 32 gene malignancy-risk signature.

Table: TOP2A and BubR1 in IDCs, high-risk and low-risk normal breast tissues.

Archival specimen type	Average # of TDLUs evaluated/ specimen (Range)	Mean TOP2A index (%) (Range)	Mean BubR1 expression score (H-score) (Range)
IDC (N=6)	Not applicable	26.7 (15-35)	149.0 (80-200)
"High-risk normal breast tissue"	33 (19-39)	11.4 (2-30)	64.4 (33-113)
Low-risk normal breast tissue"	24 (17-35)	1.8 (1-3)	16.6 (10-22)

Conclusions: Topoisomerase 2 alpha and BubR1 are novel breast cancer risk genes that can be used to determine risk of malignancy in benign breast tissue. We are further investigating the clinical utility of these markers on larger cohorts of benign breast tissues.

251 Are Columnar Cell Alteration and Sclerosing Adenosis, Independent Risk Markers for Breast Cancer?

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Background: Previous studies have found that SA carries a 1.5 to 3.7 times increased risk of developing invasive cancer compared to those without SA. This relative risk (RR) may increase to 5.5, translating to a 1.2% risk per year of cancer, if atypia is associated with the SA. On the other hand, columnar cell alteration (CCA) is noted to be associated with more worrisome lesions (ADH, DCIS or invasive cancer) in 25-30%

of cases, but the risk association with breast cancer is still unknown.

Design: SA and CCA were assessed in 9344 women in the Mayo Benign Breast Disease Cohort who underwent excisional breast biopsy between 1967-1991. Sclerosing adenosis is defined as a combination of adenosis (epithelial and myoepithelial proliferation of lobules) and stromal sclerosis. Columnar cell alteration is characterized by the presence of columnar-shaped epithelial cells lining enlarged terminal-duct lobular units (columnar cell change and hyperplasia without cytologic atypia). Heterogeneity of breast cancer risk across levels of SA and CCA was assessed, standardized to a control population (the Iowa Surveillance, Epidemiology, and End Results registry). Each factor (SA and CCA) was evaluated separately initially and also as a combined entity for association. Analyses adjusted for age at biopsy, histology (non-proliferative disease [NP], proliferative disease without atypia [PDWA] and atypical hyperplasia [AH], both ductal and lobular) and lobular involution (none, partial, complete).

Results: SA and CCA were identified in 2350 (25%) and 2025 (22%), respectively. SA and CCA were both associated with presence of proliferative disease, lower levels of involution and later age at BBD diagnosis (p<0.001 for each). After adjustment for these variables, no association was found between CCA and risk of breast cancer (p=0.54). In contrast, women with SA had increased risk compared to those without (RR=1.41, 95% CI 1.15-1.71, p=0.003). No significant heterogeneity of risk was observed when jointly examining the two factors (presence of either CCA and SA vs. neither, p=0.17; presence of both CCA and SA vs. not, p=0.28).

Conclusions: Sclerosing adenosis is an independent breast cancer risk factor, conferring a RR of 1.41; whereas CCA does not independently alter risk.

252 Diagnostic Utility of SNAIL in Metaplastic Breast Carcinoma

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Background: Metaplastic breast carcinoma (MBC) is a rare subtype of breast cancer characterized by coexistence of carcinomatous and sarcomatous components. SNAIL is a nuclear transcription factor that is incriminated in the transition of epithelial to mesenchymal differentiation of breast cancer. Aberrant SNAIL expression results in loss of expression of the cell adhesion molecule E-cadherin, an event associated with changes in epithelial architecture and invasive growth. The aim of our study is to identify the use of SNAIL in addition to traditional immunohistochemical (IHC) in accurate classification of MBC.

Design: Retrospective review of 35 cases of MBC from January 1997 to September 2007 is performed. Clinicopathologic information is obtained. A control group of 26 spindle cell lesions (4 myofibroblastomas, 14 phyllodes tumors, 8 pseudoangiomatous stromal hyperplasias) were used. IHC study was performed, using SNAIL (polyclonal; 1/500; DAKO, Carpinteria, CA), p63, EGFR, OSCAR, and wide spectrum cytokeratin (WS-KER). Scoring used <1% as negative for all markers.

Results:

Clinicopathologic factors of MBC patients

Age	Range 32-90	Mean age 62.7
Histologic grade	Low grade	8 (22.9%)
	High grade	27 (77.1%)
Histologic subtypes	Spindle cell	21 (60%)
	Mixed squamous and spindle cell	5 (14.3%)
	Squamous	4 (11.4%)
	Matrix-producing	3 (8.6%)
	Adenosquamous	2 (5.7%)
Tumor diameter (cm)	Range 0.8-24	Mean 5.0
Receptor Status	ER positive	2 (5.7%)
	PR positive	1 (2.8%)
	Her2 positive	0
Lymph node status	Positive	4 (12%)
	Negative	31 (88%)
Recurrence	Local	3 (8.6%)
	Distant	7 (20%)
Disease free survival	1 year	72%
	3 years	55%

Sensitivity, Specificity, Positive (PPV) and Negative (NPV) Predictive Values of the different immunomarkers (%)

Markers	Sensitivity	Specificity	PPV	NPV
SNAIL (61)	100	3.8	58.3	100
p63 (61)	68.6	100	100	70.3
EGFR (61)	100	19.2	62.5	100
OSCAR (61)	85.7	92.3	93.8	82.8
WS-KER (61)	77.1	100	100	76.4

Conclusions: MBC tend to be high grade, triple-negative (ER-, PR-, Her2-) carcinomas with few axillary lymph node metastases. SNAIL and EGFR are sensitive markers with low specificity for detecting MBC. p63 and WS-KER are more specific, with moderate sensitivity. OSCAR is both sensitive and specific. Therefore, a combination of p63, OSCAR and WS-KER are very useful in the work-up of MBC.

253 Does Estrogen Receptor Alpha (ESR1) Specific MicroRNAs Expression Correlate with ESR1 Transcript Levels in Breast Cancer?

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Background: Molecular mechanisms behind down-regulation of estrogen receptor in breast cancer are not well known. Recently estrogen receptor alpha (ESR1) specific microRNAs (miRNA) have been identified. MicroRNAs have a regulatory effect on their target genes . We analyzed expression of miRNAs that target ESR1 in breast cancer samples.

Design: Different morphologically recognized subtypes of breast cancer were evaluated: apocrine (n=7), metaplastic (n=14), medullary (n=14), tubular (n=6), lobular (n=6), ER positive IDC (n=24) and ER negative IDC (n=15). Immunohistochemistry for ER

alpha, AR, P63, GCFP was performed to validate the morphologic subtypes. RNA was isolated from microdissected tumor cells from the paraffin embedded blocks. Real-time PCR was performed for ESR1 and three ESR1-specfic miRNA; has-mir-7d, 22 and 206. RPL2, GAPDH and PPIA were used as control housekeeping genes.

Results: hsa-mir-7d and 22 had significantly lower expression level in ER positive compared to ER negative tumors. hsa-mir 206 was rarely expressed in ER positive breast cancer. Although ESR-1 transcript copy number was significantly different between ER negative and ER positive tumors it didn't show a quantitative correlation with miRNA level.

Conclusions: ESR1 specific miRNAs are more often expressed and show a higher transcript copies in ER negative breast cancers. They might contribute to down-regulation of estrogen receptor in ER negative breast cancers.

254 Loss of 16q in High Grade Breast Cancer Is Associated with ER Status: Evidence for Progression in Luminal Tumours?

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Background: Loss of the long arm of chromosome 16 (16q) is a common phenomenon in low grade/grade I (GI) invasive ductal carcinomas of no special type (IDC-NSTs) of the breast, whereas this event is uncommonly seen in high grade/grade III (GIII) IDC-NSTs. Together with data on the pathology and genetics of breast cancer recurrences, this has led to the concept that GI and GIII breast cancers evolve through distinct genetic pathways and that progression from GI to GIII is an uncommon biological phenomenon. Our aim was to define the genetic profiles of GIII IDC-NSTs with and without 16q loss.

Design: Frozen sections of 93 GIII IDC-NSTs were microdissected, DNA was extracted and subjected to microarray-based comparative genomic hybridisation analysis using a 32K tiling path bacterial artificial chromosome (BAC) array platform, which has a resolution of 50kb. We compared the genomic profiles of GIII tumours with 16q whole arm loss (16qWL) in the groups of oestrogen receptor (ER) positive (ER+) and negative (ER-) cancers.

Results: We demonstrate that 36.5% of GIII breast cancers display 16qWL. This genetic aberration was associated with ER expression and luminal phenotype. ER positive GIII IDC-NSTs with 16qWL displayed significantly higher genomic instability than ER positive IDC-NSTs without 16qWL. Furthermore, ER positive and ER negative IDC-NSTs stratified according to the presence of 16qWL harboured distinct patterns of genetic aberrations. Interestingly, ER+/ 16qWL tumours displayed genetic features usually found in tumours with homologous DNA repair defects (ie sawtooth genomic profiles) and significantly more frequently displayed loss of *BRCA2* than the remaining ER+ cancers.

Conclusions: Our results demonstrate that only a minority of GIII IDC-NSTs harbour 16qWL, confirming that progression from low to high grade breast cancer is an uncommon phenomenon. 16qWL was associated with ER-positivity and luminal phenotype. Given that GI cancer are consistently positive for ER and harbour a luminal phenotype, our results suggest that if progression from GI to GIII breast cancer happens may preferentially occur in breast cancers of luminal phenotype.

255 Molecular Profiling of Grade III Invasive Ductal Breast Cancers Identifies *PPM1D* Amplification as a Therapeutic Target in Luminal and HER2 Tumours

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Background: Grade III (GIII) breast cancer encompasses a heterogeneous group of cancers with aggressive clinical behaviour. Our aims were to characterise the molecular genetic profiles of GIII invasive ductal carcinomas of no special type (IDC-NSTs) using high resolution microarray-based comparative genomic hybridisation (aCGH), and to identify recurrent amplicons harbouring putative therapeutic targets associated with luminal, HER2 and basal-like phenotypes.

Design: 95 grade III-IDC-NSTs were classified into luminal, HER2 and basal-like subgroups using a previously validated immunohistochemical panel. Tumour samples were microdissected and subjected to aCGH using a tiling path 32K bacterial artificial chromosome array platform. Expression of genes pertaining to selected amplicons was investigated using quantitative real-time PCR and gene silencing was performed using previously validated short hairpin RNA (shRNA) constructs and a specific small molecule inhibitor.

Results: We demonstrate that basal-like and HER2 tumours are characterised by 'sawtooth' and 'firestorm' genetic patterns, respectively; whereas luminal cancers displayed a ore heterogeneous combination of genetic patterns. *PPM1D* gene amplification (17q23.2) was found in 20% and 8% of HER2 and luminal cancers, respectively. PPM1D mRNA levels were significantly higher in cases harbouring *PPM1D* gene amplification. Silencing of PPM1D by shRNA inhibition and by a specific PPM1D phosphatase inhibitor in a panel of cell lines resulted in selective loss of viability in tumour cell lines harbouring the 17q23.2 amplification.

Conclusions: Our results demonstrate the power of aCGH analysis in unravelling the genetic profiles of specific subgroups of cancers and for the identification of novel therapeutic targets. PPM1D may be an additional therapeutic target for a subgroup of patients with HER2 and luminal breast cancers.

256 Y-Box-Binding Protein-1 Expression Is Associated with Suppressed E-Cadherin Levels and Poor Prognosis in Breast Cancer

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Background: YB-1 is a transcription and translation regulatory DNA/RNA binding protein previously shown to be associated with cancer aggressiveness. We showed in unpublished *in vitro* data that YB-1 overexpression in the MCF-10A breast cell line results in suppression of proliferation in combination with a switch to a mesenchymal phenotype dependent on Ras overexpression. This is mediated at least in part through translational upregulation of Snail levels resulting in increased E-cadherin. We therefore wished to investigate this result in primary tumor tissue, and identify possible prognostic implications.

Design: Using a tissue microarray (TMA) consisting of 143 interpretable cases in duplicate cores of *in situ* and invasive breast cancer, with associated clinical follow-up data for 20 years, we performed immunohistochemistry (IHC) for YB-1 and E-cadherin. Immunofluorescence (IF) for the same markers was done on whole sections of normal breast tissue, ductal carcinoma in-situ (DCIS) and ductal carcinoma. YB-1 cytoplasmic and nuclear staining was scored separately (0-2+) while only membranous E-cadherin expression was scored (0-2+).

Results: Consistent with our *in vitro* data of MCF-10A cells without Ras overexpression, normal breast epithelium shows co-expression of YB-1 and E-cadherin by IF. However, far less co-expression was seen in invasive ductal carcinoma. Interestingly, several cases of DCIS showed disseminated tumor cells in subjacent stroma that were positive for YB-1 but negative for E-cadherin. The IHC of the TMA again showed a statistically significant inverse correlation between YB-1 and E-cadherin expression levels in 65% of breast cancer specimens (Fisher's exact test, p=0.033). There was no significant association between YB-1 expression alone and survival, and only a trend towards increased survival with high E-cadherin expression alone. However, compared to the cases with low YB-1/high E-cadherin, high YB-1/low E-cadherin expression strongly correlated with a significant decrease in breast cancer-related survival (p=0.0143).

Conclusions: These findings suggest that high YB-1 expression is associated with loss of E-cadherin in invasive and therefore potentially metastatic breast cancer cells, and is indicative of poor clinical outcome.

257 Pleomorphic Carcinoma of the Breast: Is Severe Pleomorphism Associated with a Worse Clinical Outcome?

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Background: The WHO classification of tumors of the breast includes a rare variant of high-grade ductal carcinoma termed pleomorphic carcinoma. These tumors characteristically contain multinucleated tumor giant cells and contain marked pleomorphism (>6-fold variation in nuclear size) at the highest end of the spectrum of grade 3 invasive ductal carcinomas. Although these tumors are reported to have an aggressive behavior, the WHO classification allows a focal spindle cell component, and it is unclear whether invasive carcinomas with severe pleomorphism without a metaplastic component have a worse clinical outcome than other high-grade invasive ductal carcinomas.

Design: We examined the clinicopathologic features of 37 cases of invasive carcinoma with severe pleomorphism (>6-fold variation in nuclear size), with or without a spindle cell component. Only cases with a striking degree of pleomorphism were included. Patients with invasive pleomorphic lobular carcinoma and those without at least a tissue biopsy prior to chemotherapy were excluded.

Results: Patients ranged in age from 23 to 78 yrs (median 49 yrs). Frequent histologic findings included multinucleated tumor giant cells in all 37 tumors, necrosis in 28 (patchy in 26 and extensive in 2), and at least a partial lymphocytic response in 26. The mitotic rate was <20 per 10 hpf in 6 tumors, between 20 and 50 in 19 tumors, and >50 in 12. A focal spindle cell component (<25%) was present in 14 tumors (38%). Fourteen cases had associated DCIS, and 18 had positive axillary lymph nodes. Clinical follow-up was available for 36 patients (median 17 months). Patients with a spindle cell component appeared to have decreased survival compared to those without a spindle cell component. Mean overall survival for patients with and without a spindle cell component was 41 ± 9 mos and 65 ± 6 mos, respectively. Overall five-year survival for patients with and without a spindle cell component was 38% ± 15% and 89% ± 7%, respectively.

Conclusions: Pleomorphic carcinoma as defined by the WHO classification is not a specific subtype of breast carcinoma. The distinctly aggressive clinical course appears limited to the subgroup of pleomorphic carcinomas with a spindle cell component. Severe pleomorphism by itself appears not to be prognostically significant.

258 Mucocele-Like Lesions of Breast with No or Minimal Epithelial Atypia on Core Biopsy: To Excise or Not?

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Background: Mucocele-like lesions (MLLs) of breast could be recognized on core biopsy (CB) by presence of mucin-filled ducts with associated extravasated mucin within surrounding parenchyma. Although some recommend surgical excision for radiologically suspicious mass-forming lesions and lesions associated with epithelial atypia on CB, there is limited data regarding whether cases with little or no epithelial atypia should be excised.

Design: Fifty-two breast CB samples between 2000 and 2008 coded as mucin, extravasation, or mucocele were identified in our departmental database. Thirty-five cases were excluded from the study due to diagnosis of concurrent invasive mammary carcinoma (n=13), ductal carcinoma in situ (n=7), or atypical ductal hyperplasia (ADH) bordering on ductal carcinoma in situ (n=2) on the CB samples, history of

ipsilateral breast cancer (n=8), no clinical follow-up (n=3), or absence of extravasated mucin (n=2) upon review of the pathology report and clinical history. The remaining 17 MLL cases had minimal to no epithelial atypia on the CB and were the subjects of this study. Slides of CB were evaluated to confirm the diagnosis. Ultrasound-guided (16- or 18-gauge) CB was performed for the 2 mass lesions, and stereotactic-guided (9-gauge) CB was performed for the calcifications with or without a mass in 15 cases. Clinical, radiologic, and pathologic data were reviewed and correlated with results of excision, if performed.

Results: Of 17 MLL cases with no or minimal epithelial atypia on CB, 6 had surgical excision and 11 were monitored by imaging studies. The table illustrates radiologic data and histology of CB samples. Residual MLL was demonstrated in 3 out of 6 excised cases, and minimal epithelial atypia was still present in 2 cases. However, no upgrades to a higher risk lesion were noted upon excision. No significant change in lesion characteristics was observed in cases without excision (median follow-up of 11 months). Of note, 3 of 6 excised cases and 3 of 11 cases in the follow-up group had a history of contralateral breast carcinoma.

Radiologic and histologic characteristics of MLLs on CB

	Imaging data			MLL associated with calcifications	CB diagnosis	
Procedure, number	Mass	Calcifications	Mass with calcifications	Number (%)	No atypia	Atypia
Excision, 6	1	2	3	5 (83)	4	1 minimal ADH; 1 columnar cell lesion w/ atypia
Clinical monitoring, 11	1	7	3	9 (82)	8	2 minimal ADH; 1 atypical lobular hyperplasia

Conclusions: Follow-up alone may be appropriate when MLL has no or minimal epithelial atypia on CB.

259   Androgen Receptor Expression in Breast Cancer Molecular Classes: An Immunohistochemical Study of 190 Consecutive Cases
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Background: Dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) have growth inhibitory effects on estrogen receptor (ER) and progesterone receptor (PR) negative breast cancer cell lines that demonstrate androgen receptor (AR) expression. These laboratory findings may be translated into inexpensive alternative therapies for ER-/PR-invasive breast cancers (IBCs). Our aim was to systematically evaluate AR expression by immunohistochemistry (IHC) in breast cancer molecular classes.
Design: AR (clone AR441, Dako) expression was analyzed on 190 IBCs represented with 3-fold redundancy on tissue microarrays. The IBCs were classified using IHC surrogate (to molecular classes) markers-ER, PR and HER2. ER and PR were scored using a semi-quantitative H-score method with a dynamic range of 0-300. HER2 was considered positive only if 3+ by IHC or unequivocally amplified by FISH. The tumors were divided in 6 groups as follows: Luminal A (**LUMA**; ER score 200 or higher, HER2 negative), Luminal B (**LUMB**; ER score 11-199 or PR >10, HER2 negative), Triple Negative (**TN**; ER and PR score 10 or less, HER2 negative), **ERBB2** (ER and PR score 10 or less, HER2 positive), Luminal A-HER2 Hybrid (**LAHH**; ER score 200 or higher, HER2 positive), Luminal B-HER2 Hybrid (**LBHH**; ER score 11-199 or PR >10, HER2 positive). AR expression was also semiquantitated using an H-score method and a score >10 was considered as positive.
Results: Of the 190 consecutive IBCs, 152 (80%) were positive and 38 (20%) were negative for AR. The AR expression with respect to molecular classes was as follows:

	AR+	AR negative	TOTAL
LUMA	103 (96%)	4 (4%)	107
LUMB	24 (86%)	4 (14%)	28
TN	3 (10%)	27 (90%)	30
ERBB2	5 (63%)	3 (37%)	8
LAHH	9 (100%)	0 (0%)	9
LBHH	8 (100%)	0 (0%)	8
TOTAL	152	38	190

Of the 8 ER-/PR-/AR+ tumors (5 of ERBB2 and 3 of TN group), 6 demonstrated apocrine differentiation.
Conclusions: Molecular classes LUMA, LUMB, LAHH, LBHH (i.e. ER+ tumors) demonstrate AR expression in the majority of cases. TN tumors show AR expression only in a subset of tumors that demonstrate apocrine differentiation. A vast majority of ERBB2 tumors are AR positive due to overwhelming presence of apocrine differentiation in these tumors. AR targeted therapy in ER-/PR- tumors may provide an inexpensive alternative to usual high dose chemotherapy with or without trastuzumab.

260   Performance of Automated Brightfield Dual Color In Situ Hybridization (ISH) for Detection of *HER2* **Gene Amplification in Breast Carcinoma**
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Background: *HER2* Dual color *HER2* fluorescence in situ hybridization (FISH) procedures are labor intensive methods that require highly skilled technologists and specialized fluorescence microscopy. We evaluated a fully automated brightfield dual color ISH method for detection of *HER2* gene and chromosome 17 centromere (CEN 17) against a *HER2* FISH reference standard.
Design: Dual color fully automated bright field ISH using DNP-labeled *HER2* DNA probe and a DNP-labeled CEN 17 oligoprobe were sequentially detected via silver in

situ hybridization (SISH) (black signal) and chromogenic in situ hybridization (CISH) using fast red and naphthol phosphate reaction (red signal), respectively. Specificity of the assay was confirmed with formalin-fixed, paraffin-embedded xenograft tumors, MCF7 and BT-474. Concordance of the brightfield dual color ISH assay with dual color FISH (PathVysion; Abbott Molecular) was assessed for 94 breast carcinomas.
Results: Consensus concordance (historical method) was 98.9% (Kappa = 0.9736, 95%CI = 0.9222 – 1.0000); sensitivity 96.3%, specificity 100%. Individual concordance ranged from 97.8% (Simple Kappa = 0.9466, 95%CI = 0.8736 – 1.0000) to 100% (Simple Kappa = 01.0000, 95%CI = 1.0000-1.0000). For ASCO/CAP scoring method and equivocal cases included, consensus concordance rate was 95.7% (Simple Kappa = 0.8993%, 95%CI = 0.8068-0.9919); individual scorers 92.5% (Kappa = 0.8275, 95%CI = 0.7102-0.9448) to 95.7% (Kappa = 0.9069, 95%CI = 0.8206 - 0.9933). When equivocal cases were excluded the consensus concordance was 100% (Kappa = 1.0000%, 95%CI = 1.0000-1.0000) (sensitivity 100%, specificity 100%; individual concordance 97.7% (Kappa = 0.9442, 95%CI = 0.8678-1.0000) to 100% (Kappa = 1.0000, 95%CI = 1.0000-1.0000).
Conclusions: Excellent concordance was documented between the fully automated *HER2* brightfield ISH assay and FISH. *HER2* gene status can be accurately assessed within the context of tissue morphology using a fully automated brightfield dual color ISH application.

261   Breast Carcinoma with Positive Lymph Node Status: Assessment of Clinicopathological Variables with Prognostic Implications
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Background: Axillary lymph node (LN) status is the most powerful prognostic factor in breast cancer (BC). The aim of the current study was to investigate the clinicopathologic and immunohistochemical (IHC) features with potential prognostic significance, such as histologic grade (HG), tumor size, Bcl2, Ki67, ER, PgR, Her2, p53 and tumor phenotypes in a series of BC with LN metastasis.
Design: Surgical specimens from 763 patients with invasive BC and axillary lymphadenectomy were studied. Median clinical follow-up was 78 months (range 12-245). Histologic grade (HG) was assessed (Nottingham). IHC staining: Bcl2 (cut-of 50%), ER (cut-of 10%), PgR (cut-off 10%), Ki67 (cut-off 20%), p53 (cut-off 20%) and Her2 (2+ and <30% 3+ confirmed by FISH). Tumors were classified according to the immunophenotype as luminal "low-risk" (Ki67/p53 <20%), luminal "high-risk" (Ki67/p53 >20%), Her2-positive and triple-negative (ER/PgR/Her2-negative). Cases were stratified according to the LN status (N0, N1-3, N>3). Significant associations were identified using Chi-square and Fisher's exact test. Survival curves were calculated by the Kaplan-Meier method (log rank test). Multivariate analysis was determined by Cox's proportional hazard model.
Results: According to the LN status, 66% were N0, 22% N1-3, and 14% N>3. Based on IHC findings 48% tumors were of luminal "low risk" subtype, 15% luminal "high risk", 20% Her2-positive and 17% triple negative. HG was G1 in 19%, G2 in 36% and G3 in 45%. Increased Ki67 was observed in 39% of tumors, p53 was positive in 22% and low Bcl2 in 38%. LN status correlated with HG, tumor size, Bcl2 and immunophenotype (all p<0.05). Poorer survival was seen for patients with higher number of positive LN, larger tumors, of HG 3, with high Ki67 and low level of Bcl2 (all p<0.05). Among patients with N>3 BCs, better survival was observed for those with tumors of luminal "low risk" compared to those with luminal "high risk", Her2-positive or triple-negative phenotypes (p=0.007).
Conclusions: Our study showed a better prognosis for patients with BC of luminal "low risk" immunophenotype and N>3 axillary status compared to those with other more aggressive phenotypes, at the same clinical stage. In addition, analysis of Ki67 and Bcl2 proportion relevant prognostic information in this group of patients. Supported by grant FIS PI061488.

262   HER2/neu Status on Pre and Post Neoadjuvant Chemotherapy
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Background: Neoadjuvant chemotherapy is administered in locally advanced breast cancer and in some cases of resectable cancers. The selection of chemotherapeutic agents and Trastuzumab in this setting is based on the expression of biological markers tested on core needle biopsies (CNB). In this study, we examined the possible effect of neoadjuvant treatment on the expression of HER2/neu by assessing the concordance between HER2/neu tested prior and following treatment.
Design: We assessed HER2/*neu* status on breast core biopsies in a cohort of 53 cases registered at the Odette Cancer Centre in the locally advanced breast cancer clinic before treatment was initiated. All CNB were evaluated for HER2/*neu* oncoprotein overexpression using immunohistochemistry (IHC) and gene amplification using florescence in situ hybridization (FISH). The subsequent resection specimens were evaluated by IHC and equivocal cases were assessed by FISH. The neoadjuvant regimen in these patients included anthracycline based chemotherapy (doxorubicin or epirubicin) and in patients with ER/PR negative tumors taxanes (paclitaxel, docetaxel). None of the patients received neoadjuvant trastuzumab.
Results: In 45/53 cases HER2/neu status was available on the resection specimens (5 cases had no residual tumor, 2 cases had the resection in another hospital and 1 case progressed and remained unresectable). CNB were HER2/*neu* positive in 14/45 (31.1%) cases by IHC and in 15/45 (33.3%) by FISH. Resection specimens were HER2/neu positive in 12/45 (26.7%). Overall IHC-CNB correlated with IHC-resection in 39/45 (86.6%). There were 6 discordant cases: 4 IHC-CNB positive/IHC-resection negative and 2 IHC-CNB negative/IHC-resection positive. Of note, 3/4 IHC-CNB positive/IHC-resection negative cases were FISH negative and the fourth case demonstrated low level of amplification. Overall FISH-CNB correlated with IHC-resection in 41/45 (91.1%). There were 4 discordant cases, all FISH-CNB positive/IHC-resection negative. Two of 4 discordant cases demonstrated low level of amplification.

Conclusions: This group of aggressive breast cancer shows a relatively high percentage of HER2/neu positivity (30%). There is a high level of concordance of HER2/neu status on CNB and tumors post neoadjuvant treatment**.** We observed a change in HER2/neu status in about 10% of the cases post chemotherapy which may be a result of chemotherapy effect or tumor heterogeneity. Retesting residual tumor for HER2/neu post treatment is therefore recommended in this setting.

263 Analysis of CD34, CD117, and Ki-67 Expression in 33 Cases of Phyllodes Tumor of the Breast

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Background: Phyllodes tumor of the breast is an uncommon stromal neoplasm which can behave in a benign or malignant manner. The aim of this study was to evaluate the expression of CD34, CD117 and Ki-67 in phyllodes tumor (PT) of the breast, and attempt to correlate the staining pattern with tumor grade by morphology.

Design: Immunohistochemical expression of CD117, CD34 and Ki-67 was studied on formalin-fixed, paraffin-embedded archival tissue material from 33 cases of phyllodes tumor of the breast retrieved from the California Tumor Tissue Registry (CTTR). All 33 cases were reviewed by the authors and classified into three categories based on the current WHO criteria. Histologically, there were 21 low grade, 6 borderline, and 6 malignant (high grade) tumors. All patients were female and ranged from 13 to 86 years of age.

Results: All six histologically malignant PTs were positive for CD117 (100%), but only one marked with CD34 (16.67%). The majority of histologically low grade PTs were positive for CD34 (18/21, 85.7%) while CD117 was positive in fewer cases (7/21, 33.3%). The histologically borderline tumors were positive for CD34 in all cases (6/6, 100%) while CD117 was positive in most cases (5/6, 83.3%). Ki-67 staining ranged from 4%-20% in malignant lesions, <1%-10% in benign PTs and 2%-15% in borderline PTs.

Conclusions: Histologically benign or low-grade PT showed frequent expression of CD34 while CD117 was expressed less often. The morphologically malignant PTs, however, showed a relatively low CD34 expression but all expressed CD117. These findings are in agreement with previous reports of CD34 as a useful marker for histologically benign but not malignant PT. Additionally, there appears to be increasing CD117 expression with increasing degree of malignancy. In our study, the Ki-67 proliferative index did not distinguish morphologically benign from malignant PT. However, additional study with more complete follow-up information is necessary to determine if there is a correlation between proliferative index and biologic behavior.

264 Expression of Gamma-Laminin, Cytokeratin 5 and Cytokeratin 5/6 in Metaplastic and Basal-like Breast Carcinomas

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Background: Basal-like (BL) and metaplastic (MPC) breast carcinomas are part of the "triple-negative" (TN) group of breast carcinomas. The triple-negative status connotes negativity for estrogen and progesterone receptors and HER2 oncoprotein. Identification of the basal phenotype in TN tumor requires the demonstration of cytokeratin (CK) 5/6 and/or epidermal growth factor receptor (EGFR). A recent paper has described the superiority of gamma-laminin over CK 5/6 in the identification of BL and MPC. We have recently demonstrated the dramatic superiority of CK 5 over CK 5/6 for identification of BL tumors (Mod Pathol 2008;21 [suppl 1]:23A-Abstract 92). This study compares the efficiencies of CK 5/6, CK 5 and gamma-laminin to identify BL and MPC.

Design: Nine MPC carcinomas (previously positive for 2 of the following 4 markers: CK 5/6, CK 14, CK 17, EGFR) and 14 BL were used as a basis for this study. All tumors were immunostained with CK 5/6 (D5 and 16B4, Ventana Medical Systems), CK 5 (XM26, Vision Biosystems), EGFR (3C6, Ventana Medical Systems) and gamma-laminin (D4B5, Chemicon International). EGFR was scored analogous to current HER2 guidelines. Other antibodies were scored numerically based on the percent of cells stained: 1= 1-25%; 2=26-50%; 3= 51-75%; 4 >75%.

Results: All BL and MPC were CK 5/6, CK 5, and EGFR positive. All CK 5 positive cases had more than 75% cells positive (score=4) diffusely. Immunostaining for CK 5/6 was extremely variable, with less than half of the cases demonstrating a score of at least 3, and only 5 scoring a 4. Gamma-laminin immunostaining, similar to CK 5/6, was quite variable, with only 7 cases scoring a 4.

Expression of Gamma-Laminin, CK 5, and CK 5/6.

	Gamma-Laminin	CK5	CK 5/6
Metaplastic			
1	2	4	4
2	2	4	2
3	1	4	3
4	3	4	1
5	4	4	4
6	4	4	4
7	4	4	4
8	1	4	1
9	2	4	4
Basal-like			
1	3	4	3
2	3	4	2
3	1	4	3
4	3	4	3
5	1	4	1
6	1	4	1
7	3	4	1
8	3	4	1
9	4	4	1
10	4	4	3
11	4	4	Not done
12	1	4	1
13	2	4	1
14	4	4	2

Conclusions: Cytokeratin 5 is superior to gamma-laminin and CK 5/6 for identification of the BL and MPC phenotypes of TN tumors. The intensity and percent of cells immunostaining for CK5 is substantially greater compared to laminin and CK 5/6. Basal phenotype markers are useful in identifying MPC.

265 Lobular Carcinoma In-Situ/Atypical Lobular Hyperplasia on Breast Biopsies: Does It Warrent Surgical Excision?

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Background: Atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS) are associated with increased risk of developing invasive breast carcinoma in either breast. These changes are often incidental findings in breast core needle biopsies (CNBs). The management of these changes in otherwise benign breast biopsies remains controversial. In this study, we reviewed histologic features of ALH and LCIS in CNBs and correlated biopsy findings with those in the follow-up surgical excisions.

Design: We retrieved 2228 breast CNBs from the surgical pathology files between 2003-2008 and identified 35 cases having a diagnosis of ALH or LCIS (1.6%). Seven cases were excluded due to the presence of more severe lesions on the CNBs which mandated excision. The remaining 28 cases contained only ALH or LCIS and otherwise benign breast tissue; 13 had surgical excision follow up. These cases were retrospectively reviewed for extent of atypical change, ductal spread, and association with microcalcifications. These data were correlated with the follow-up surgical excision findings.

Results: The results are shown in the table below. Five out of 13 cases (38%) were upgraded to a diagnosis of carcinoma at excision. Upon retrospective review, all 5 cases had involvement of ducts by ALH/LCIS and 4 cases had extensive ALH/LCIS in CNB. The coexistence of extensive ALH/LCIS and ALH/LCIS ductal involvement in 7 CNBs was correlated with carcinoma (4/7) or extensive LCIS (3/7) in subsequent excision. ALH/LCIS with/without microcalcifications in CNBs didn't seem to be significant.

Patient	Needle biopsy diagnosis	Microcalcifications	Involvement of Ducts	Extensive (>2 TDLU)	Excision Diagnosis
1	LCIS	Present	Present	Yes	ILC
2	LCIS	Absent	Absent	No	Benign
3	LCIS	Present	Present	Yes	Extensive LCIS
4	LCIS	Absent	Present	No	IDC
5	LCIS	Absent	Present	Yes	DCIS
6	ALH	Present	Present	Yes	IDC and DCIS
7	ALH	Present	Absent	No	Benign
8	LCIS and ALH	Absent	Present	Yes	LCIS
9	LCIS	Present	Absent	Yes	Benign
10	LCIS	Absent	Present	Yes	Extensive LCIS
11	LCIS	Present	Present	Yes	IDC and ILC
12	ALH	Present	Present	No	ALH
13	ALH	Absent	Present	No	LCIS

TDLU – terminal ductal lobular unit, DCIS – ductal carcinoma in-situ, ILC – invasive lobular carcinoma, IDC – invasive ductal carcinoma

Conclusions: The histologic diagnosis of ALH/LCIS in breast CNBs should include the extent of the lesion and the presence or absence of ductal involvement. If ALH/LCIS is extensive and involves ducts, excision is warranted.

266 Magenetic Resonance Imaging of the Breast: Radiologic-Pathologic Correlation

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Background: Magnetic Resonance Imaging (MRI) of the breast has been increasingly used in diagnosing occult cancers, for staging purposes and in assessing response to neoadjuvant chemo/hormonal therapy. MRI's high cost and low specificity restricted its use in routine breast cancer screening. This pilot study focuses on the role of MRI in (1) detecting multifocal/multicentric disease and (2) its utility in the management of patients with newly diganosed breast cancer.

Design: Pathology department database and electronic patients' medical record were searched to select patients who had undergone mastectomy and who had pre-operative breast MRI examination at Loyola University Chicago Medical Center during the

period July 2007 through June 2008. We systematically reviewed the accuracy of MRI in detecting multifocal and / or multicentric cancers not identified on conventional imaging (mammography and ultrasound). We estimated positive predictive value (PPV) and sensitivity, and also the proportion of women whose treatment were altered due to MRI findings. Interclass Correlation Coefficient is used for statistical analysis.
Results: There were a total of 59 patients who underwent mastectomy as a definitive treatment and who had pre-operative MRI of the breast as part of the management of breat cancer. Seven patients were excluded due to pre-operative neoadjuvant therapy. In contrast to mammography and ultrasound imaging, MRI detected additional lesions in 16 patients (27 %) which affected the surgical management. Pathological lesion size ranged from 0.4 to 6.2 cm (median 1.5 cm).In this analysis, positive predictive value for MRI is calculated as 93% with a sensitivity of 91%. False positive rate is 6% and false negative rate is 8%. There is a statistically significant correlation between MRI findings and pathological findings (p<0.003).
Conclusions: MRI imaging results in mastectomy as a definitive treatment in 27% of women by identifying additional cancers. Breast MRI significantly increases rate of mastectomy and the use of systemic therapy because of its accuracy in evaluating the extent of the disease. MRI of the breast is especially useful in detecting unsuspected mammographically and ultrasonologically occult ductal carcinoma in situ. Randomized clinical trials are needed to determine the value of MRI in detecting additional tumors which changes surgical management in women with breast cancer.

267 Invasive Ductal Carcinoma with Lobular Features and Invasive Pleomorphic Lobular Carcinoma – Are They Different Entities?
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Background: Recent data suggest that the prognosis of ductal and lobular carcinoma is comparable when adjusted for tumor grade and stage; however, lobular carcinoma has perceived biologic characteristics that can affect the diagnostic approach and management. E-cadherin is helpful in differentiating invasive ductal carcinoma with lobular features (IDC-LF) from invasive pleomorphic lobular carcinoma (PLC) as they have overlapping growth pattern. We compared clinical and histopathologic characteristics and the expression of biological markers in IDC-LF and PLC.
Design: We identified 97 cases from the pathology database using natural language search for "lobular" within breast specimens. Histopathologic features and ER, PR, Her-2/neu and E-cadherin status were studied. Cases were classified as IDC-LF when E-cadherin was expressed as complete membranous stain or PLC when E-cadherin was negative.
Results: Based on E-cadherin expression, there were 51 IDC-LF and 46 PLC cases. The mean age was significantly higher in PLC (55.9 vs. 63.3, p=0.0047). The rate of screen detected cancer was slightly higher in PLC (41% vs. 54.3% p= 0.197). The type of in situ component was significantly associated with that of the invasive component (DCIS present in 64% of IDC-LF vs.0% of PLC; p<0.001 and LCIS present in 58.7% of PLC vs.7.8% of IDC-LF; p<0.001). Tumor size, nodal status, lymphovascular invasion bilaterality and multifocality were comparable between the groups (p=0.749, p=0.453, p=0.052 p=0.879 and p=0.321 respectively). Interestingly, the likelihood of ER, PR expression was not significantly different (ER+ in 90.2% of IDC-LF vs.91.3% of PLC; p=0.852; PR+ in 72.5% of IDC-LF vs.73.4% of PLC; p=0.880). Her-2/neu overexpression was demonstrated in 15.72% of IDC-LF vs. 4.3% of PLC. The lower proportion of Her2/neu overexpression in PLC approached significance (p=0.068). The likelihood of mastectomy was slightly higher in PLC (43.5% vs. 37.2%, p=0.535). With a mean follow up of 24 months (both groups), there were 3/51 locoregional recurrences and one distant recurrence (liver) in IDC-LF cases and 3/46 locoregional recurrences and one distant recurrence (bone) in PLC cases.
Conclusions: Unlike the known differences between classic lobular and ductal carcinomas, PLC does not significantly differ from IDC-LF in its biological characteristics with the exception of lower percentage of Her2/neu overexpression. Testing for E-cadherin may have limited clinical value.

268 Breast Scar Gene Expression Profiles Overlap with Tumor Stroma Response Signatures
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Background: The stroma surrounding cancer cells has emerged as an important factor in the development and subsequent behavior of breast tumors. We have previously demonstrated specific, unique stromal gene expression signatures derived from soft tissue tumors: desmoid-type fibromatosis (DTF), solitary fibrous tumors (SFT) (West RB, *et al.* 2005 PLoS Biol. 3(6):e187), and tenosynovial giant cell tumor (TGCT). We hypothesize that these tumor-associated stromal patterns are also present in normal, physiologic responses, like wound response. The goal of this study was to identify expression profiles associated with scar formation in breast tissue and to determine the overlap, if any, between breast scar formation and SFT, DFT, and TGCT (CSF1 response) signatures.
Design: We searched recent Stanford Pathology archives for formalin-fixed paraffin-embedded (FFPE) breast tissue for biopsy site change following core biopsy or lumpectomy. We chose 14 cases with post-procedure intervals ranging from 7 to 51 days. We isolated and amplified the RNA, and then performed gene expression profiling on 44K element oligonucleotide arrays (HEEBO) for each sample. We then compared the gene expression profiles of the 14 breast scar cases with a list of genes previously shown to comprise the breast cancer stromal signature associated with the DTF, SFT, and TGCT (CSF1 response) signatures.
Results: Gene expression profiles of breast scars show overexpression of a common set of genes. Breast scar expression profiles demonstrate significant overlap with the DTF and TGCT (CSF1 response) stromal signatures.
Conclusions: RNA derived from FFPE tissue can be amplified and used to identify gene expression profiles for scar tissue. Stromal responses to wound healing and stromal

responses to tumor share some elements in their expression profiles. Breast scar gene expression profiles may be useful for identifying new activation states for fibroblasts, myofibroblasts, and other stromal elements.

269 Cell Signalling Factors in Breast Tumors. Tumor Cell Distribution May Define Tumors with Different Oncogenic Pathways Activated. The Critical Role of the Pathologist in Its Evaluation
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Background: Cell signalling is a crucial cellular pathway in breast carcinomas with a myriad of oncogenic alterations involved. The cell signals converge through a few pathways like pAKT,pMAPK and mTOR.Downstream mTOR factors such as pS6,p4E-BP1 and eIF4E/eIF4G control final cap-dependent protein traslation.Because breast tumors show a clear heterogeneity,we studied whether a diffuse or patchy immunohistochemical expression of downstream mTOR factors throughout the tumors could reflect a constitutive activation of the pathway or indicate complex concomitant epigenetic and oncogenic signals.
Design: We studied 34 primary tumors and 32 lymph node metastasis.By immunohistochemistry, real time-PCR and WB we evaluated the expression of 4E-BP1,p4E-BP1,peIF4E and eIF4G.Immunohistochemistry alone was performed for pMAPK and pS6.The expression of these factors was evaluated in whole sections of tumors according to a semi-quantitative score and to their tumoral distribution.The results were correlated with levels of protein and mRNA,tumor grade and metastasis.
Results: In our series,4E-BP1,p4E-BP1,eIF4E and peIF4G showed a predominant diffuse pattern of expression while pMAPK displayed a peripheral or patchy distribution in 57% cases and pS6 showed a patchy distribution in more than 70%.Interestingly,pS6 was stronger in the invasive border.Tumors with a diffuse expression of pS6 were usually grade III (65%) and had lymph node involvement (75%).The pattern of expression was the same in the primary tumor and in its metastasis.4E-BP1 and eIF4E study by WB and RT-PCR did not show a linear correlation between protein levels,mRNA or immunohistochemistry results.
Conclusions: 4E-BP1 and eIF4E protein expression levels associate with high grade tumors and lymph node involvement.Moreover,they show a diffuse and then constitutive expression while pMAPK and pS6 show a great heterogeneity with a predominant peripherical distribution.These results support that 4E-BP1 can reflect oncogenic activation of other cellular pathways in addition to mTOR Interestingly,a pS6 diffuse pattern (constitutive mTOR activation) associates with histological grade and metastasis. Finally, these results underline the critical role of the pathologist in the evaluation of cell signaling factors and the pitfalls that can be made from frozen tissues studies because of the tumor heterogeneity of many of these markers.

270 PI3K/Akt/mTOR Pathway in HER2-positive Invasive Breast Carcinoma. An Immunohistochemical and Molecular Analysis
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Background: Increased activity of PI3K/Akt signaling pathway due to the interaction between growth factor receptors and tumor suppressor genes has been shown in a number of human cancers. Recent mutational analyses of PIK3CA (3q26.3), which encodes the PI3K catalytic subunit p110α, have suggested increasing also the PI3K activity inducing oncogenic transformation. PI3K/Akt pathway alterations in HER2-positive invasive breast carcinomas (IBC) according to the hormone receptor status have not been extensively evaluated.
Design: Tissue microarrays were constructed from pre-selected tumor areas of 185 IBC HER2-positive, determined by immunohistochemistry (IHC) and/or molecular (CISH and/or FISH in cases with <30% positive tumor cells) methods. Further IHC was performed to study the expression of p110α, phospho-Akt (Ser473) and mTOR (Ser2448), IGF1R, ER and PTEN. The data were scored semiquantitatively based on staining intensity (0-3+) and distribution (0-100%) (score 0-300). We performed a mutational analysis of PIK3CA gene G1624A (E542K) and G1633A (E545K) in exon 9 (helical domain), and A3140G (H1047R) in exon 20 (kinase domain) from 80 tumors by allelic discrimination based on real-time chemistry TaqMan MGB probes in ABIPrism 7500 Sequence Detection System (Applied Biosystems). Direct DNA sequencing at exons 9 and 20 in ABIPrism 310 confirmed all positive samples. The results were correlated to standard clinical-pathological parameters.
Results: Mean follow-up was 73 months (SD +/-52 months); 16% of patients were younger than 40 years. Tumors were more frequently >2cm in size (53%), of ductal type (96%), grade 3 (67%), showing 55% necrosis and 56% vascular invasion. ER-positive was found in 43%, loss of PTEN in 20%, increased IGF1R in 63%, p110α in 36%, p-Akt in 65% and p-mTOR in 42% cases. PIK3CA mutations were detected in 17.5% (14/80) cases, predominantly at exon 20 (10/14; 71.4%). HER2/ER-positive tumors showed lower grade, increased IGF1R and p110α, and PTEN was present (p<0.02). However, no association was observed with the levels of p-Akt, p-mTOR, PIK3CA mutations or the other clinical-pathological factors (all p>0.05).
Conclusions: IGF1R and PTEN are involved in the PI3K/Akt pathway aberrations playing a distinct role in the pathogenesis of HER2/ER-positive IBC. Apparently, PIK3CA mutations, more frequently present in exon 20, have different functional effects in this subset of tumors. Supported by grant FIS 06/1495.

271 Refining the Diagnostic Approach in the Evaluation of HER2 in Breast Cancer: A Survey of 2008 Invasive Breast Cancers Comparing a Rabbit Polyclonal Antibody, Rabbit Monoclonal Antibody with Fluorescence In Situ Hybridization
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Background: Currently, laboratory testing of HER-2 in breast cancer is the second most common predictive biomarker test performed worldwide. Yet, despite recent efforts to improve the accuracy of HER2 testing by regulatory bodies, this goal has not been accomplished. We wished to evaluate the potential utility of a recently-described HER2 rabbit monoclonal antibody alongside a well-established rabbit polyclonal antibody and a well-established FISH assay on a large cohort of breast cancers and correlate the results with FISH results as well as tumor histologic and nuclear grade.
Design: 2008 invasive breast cancer cases were included in this study. Tumors were tested with DAKO A0485 rabbit polyclonal antibody (also used in HercepTest; Dako, Carpenteria, CA, USA), rabbit monoclonal antibody (clone SP3; Thermo, Fremont, CA, USA), and an FDA-approved FISH assay (PathVysion; Abbott Molecular, DesPlaines, IL, USA). This study is a slight modification of a previously published QA program (JAMA 28;291(16):1972-7), where clinical testing for HER2 by FISH were paralleled by IHC testing as well as documentation of the Nottingham tumor grade and specifically the nuclear score.
Results: When FISH is considered the gold standard assay, the polyclonal antibody had a sensitivity of 100% and specifity of 78%, while SP3 showed 84% sensitivity and 93% specifity. While There was no significant correlation between Notthingham grade and HER-2 status, nuclear grade 1 had the most powerful (100%) negative predictive value of negative FISH results (p<0.001).
Conclusions: A few conclusions can be drawn from this study on a large cohort of patients: 1. While the recently described rabbit monoclonal antibody is more specific than the more established polyclonal antibody, it lacks from adequate sensitivity. 2. The combination of these two antibodies can accurately predict gene status by FISH and thus be considered an alternative to FISH testing to assess HER-2/neu status in patients with breast cancer. Even though using 2 antibodies for IHC testing will add to the cost of HER2 testing, it reduces the need to the much more expensive FISH test in the majority of cases. 2. FISH should remain mandatory for tumors with 2+ (equivalent) cases. 3. Tumors with nuclear grade 1 (regardless of histologic type) can be safely spared HER2 testing by either modality.

272 Mucocele-Like Lesions Diagnosed by Core Needle Biopsy of the Breast: Correlation with Surgical Excision
DGK Phillips, CJ Sailey, J Warner, OB Ioffe. Univ of MD, Baltimore, MD.
Background: Mucocele-like lesions (MLL), mucinous DCIS and invasive mucinous carcinoma represent a pathologic continuum and may coexist in the same region of the breast, thus increasing the likelihood of sampling error when they are diagnosed on image-guided core needle biopsy (CNB). Currently, excision is recommended for any MLL diagnosed on CNB although literature on this subject is limited and controversial.
Design: We have retrospectively reviewed CNBs with the diagnosis of MLL dating from 1997 to 2008 which had an excision. This review identified 12 MLLs in 11 women (age 34-85, average 52.3). When MLL was seen in association with a columnar cell lesion, we used the morphologic schema by Schnitt to classify them as columnar cell change or hyperplasia (CCC/CCH) and flat epithelial atypia (FEA). 9 (75%) lesions presented with microcalcifications (MC) and were biopsied under stereotactic guidance using an 11g vacuum-assisted probe, and 3 (25%) presented as a mass; these were biopsied under ultrasound guidance with a 14g needle.
Results: 2 of 12 (17%) cases were upgraded to malignant on excision, both were low-grade DCIS. Two of the 3 biopsies performed for a mass showed only extravasated mucin and benign breast tissue on CNB; one of these cases had a 1.6 cm focus of low-grade mucinous DCIS on excision. Overall, 1 of 7 (14%) MLLs without atypia (5 CCC and 2 mucin-only) was upgraded to malignancy on excision, and 1 of 5 (20%) MLLs with FEA and/or ADH proved malignant. 2 of 4 (50%) cases with no residual radiologic abnormality showed malignancy, whereas none of the cases with residual findings were upgraded.

CNB feature	Indication		Excision Results (number of cases)				
	MC	Mass	CCC/CCH	FEA	ADH	FEA+ADH	DCIS
Mucin Only, n=2	0	2	1	0	0	0	1
MLL+CCC/CCH, n=5	4	1	4	1	0	0	0
MLL+FEA, n=2	2	0	0	1	0	1	0
MLL+ADH, n=1	1	0	0	0	0	0	1
MLL+FEA+ADH, n=2	2	0	1	0	1	0	0

Conclusions: To the best of our knowledge, this is the largest study of surgical follow-up of benign MLLs diagnosed on CNB. Cases showing FEA and/or ADH associated with MLL were more likely to show malignancy on excision. However, we report one case of CNB showing only extravasated mucin, which revealed DCIS at surgery. MLLs presenting as a mass, although rare, were more likely to harbor malignancy. Excision should be recommended for any mucocele-like lesion, including those without an epithelial component.

273 The Role of IGF1R and PTEN/Akt Pathway in Young Patients with Breast Carcinoma According to Immunophenotypes
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Background: Breast carcinoma (BC) in women aged 40 years or younger differs in many significant ways from those arising in older patients. Stratification of BC in immunophenotypes has shown different survival of these patients. However, the relevance of growth factor receptors and tumor suppressor genes such as IGF1R (Insulin-

like growth factor 1-receptor) and PTEN, and their correlation with the Akt/Bad/mTOR pathway have not been extensively studied in this subset of patients.
Design: Formalin-fixed paraffin-embedded tissue from 146 BC in women <40 years were retrieved from the archives of the Department of Pathology at the General Hospital of Alicante (Spain). Two 1mm cores were taken from separate areas of each tumor. Four tissue microarray blocks were created. Immunohistochemistry for hormone receptors (ER/PR), HER2, Ki67, HER1, CK5/6, IGF1R, PTEN, phospho-Akt (Ser473), phospho-BAD (Ser136) and phospho-mTOR (Ser2448) were performed. Tumors were classified as (a) Luminal A (RE/RP+; HER2-; Ki67<20%); (b) Luminal B (RE/RP+; HER2-; Ki67 >20%); (c) HER2+; (d) Basal-like (ER/PR/HER2-; CK5/6 and/or HER1+). Significant associations were identified using Chi-square and Fisher´s exact test. Survival was calculated by the Kaplan-Meier method (log rank test). P value < 0.05 was considered significant.
Results: Mean age of the patients was 35 years (range 20-40 years). The distribution of immunophenotypes was as follows: 45 (30.8%) tumors were classified as Luminal A, 40 (27.4%) as Luminal B, 29 (19.9%) were HER2+ and 32 (21.9%) Basal-like. Luminal A tumors showed PTEN expression preserved (92%; p<0.000) and IGF1R overexpression (82%; p<0.000). In contrast, the Basal-like group showed more frequently loss of PTEN (21%; p<0.000), lower levels of IGF1R (68%; p<0.000) and also a trend towards an association with increased p-Bad (95%; p=0.19) and p-mTOR (53%; p=0.11). However, no significant association was found with the levels of p-Akt (p=ns). Among subtypes, better survival was observed for those patients with tumors with increased IGF1R (p=0.031) or preserved PTEN (p=0.05).
Conclusions: In our series of BC in young patients (<40 years) Luminal A is the most frequent immunophenotype containing increased expression of IGF1R and PTEN present, which in turn confer better prognosis. On the contrary, in Basal-like tumors, low IGF1R or loss of PTEN imply shorter patients' survival. Supported by grant FIS 06/1495.

274 Is the Histologic Tumor Grade an Important Prognostic Factor in Small (T1, T2) Node-Negative Breast Adenocarcinomas?
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Background: Prognosis of invasive breast cancer correlates with tumor grade (G). While reporting it as low (G1), intermediate (G2) and high grade (G3) is recommended by AJCC staging system, the TNM classification does not incorporate this data. For large tumors (T3, T4) G may be clinically irrelevant. However, the same may not hold true for small (T1, T2) tumors. This retrospective study analyzes the clinical outcome in patients with small node-negative (N0) carcinomas based on tumor G.
Design: 111 excision pathology reports from patients with T1N0 and T2N0 breast tumors and known variables were retrieved from 1995 to 2007. Tumors included 10 T1a, 23 T1b, 45 T1c, and 33 T2. Clinical follow-up (FU) ranged from 7 to 152 months (mean interval 56 months). Lymphovascular invasion (LVI), hormonal and Her-2/neu status, and FU data from Tumor Registry were analyzed based on tumor size and G.
Results: 78 T1 tumors included 15 G1, 38 G2 and 25 G3. On FU 71 (93%) patients were alive and 3 deceased without disease, one died (G1) and another was alive (G2) with disease. 33 T2 tumors included 1 G1, 16 G2 and 16 G3. On FU 27 (82%) patients were alive and 2 deceased without disease, 3 died (all ER-, including one triple-) and one was alive with disease; all 4 had G3 tumors, including two with LVI. Of T1 patients 38% and 24% had received hormonal and chemotherapy, respectively, as 33% and 67% in T2 (2 of 4 with disease had chemotherapy, and 2 did not).

Clinical Outcome by Tumor Grade in Small (T1,T2) Node-Negative Breast Adenocarcinomas

Tumor Size	T1 (N=76)*			T2 (N=33)		
	Clinical Outcome**			Clinical Outcome**		
	Without Disease	With Disease		Without Disease	With Disease	
Tumor Grade	N (%)	N (%)	p***	N (%)	N (%)	p***
G1-G2	50 (66%)	2 (3%)		17 (52%)	0 (0%)	
G3	24 (31%)	0 (0%)	0.46	12 (36%)	4 (12%)	0.04

*Excludes 2 alive, status unknown **Clinical outcome includes patients living and deceased ***p value, one-tailed Fisher exact test
Conclusions: 1) Regardless of histologic G, the overall prognosis for T1N0 and T2N0 breast adenocarcinomas is very good. 2) In this small study, there is no evidence that higher G significantly impacts the clinical outcome in T1N0 tumors. 3) In T2 tumors, our data suggests that histologic G might be prognostically significant and relevant in influencing treatment decisions.

275 Interval Breast Cancers Are Associated with More Aggressive Pathologic Characteristics Compared to Screen-Detected Cancers: A Nested Case Control Study from a Canadian Breast Screening Program
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Background: The evaluation of clinical and pathological differences between screen-detected and interval breast cancers has been limited by the failure to control for factors known to influence breast cancer biology such as age at diagnosis as well as differences in recommended screening intervals between age groups and based on family history.
Design: A case-control study nested within the participants of the population-based Nova Scotia Breast Screening Program diagnosed between ages 40-69 in the period 1991-2004 was performed. Interval cases were selected as having developed after a negative screen and prior to the recommended next screen and were validated by blinded review of the pre-diagnosis screening mammogram by 3 radiologists, 2 of whom had to agree that the screening exam was negative. Screen-detected cases were matched to intervals on a 2:1 basis by 5-year age group and recommended screening interval (40-49 yo, annual; 50-69 yo positive family history, annual; 50-69 yo negative family

history, biennial). Pathologic variables were abstracted and slides reviewed for cases with incomplete reports. Logistic regression controlling for matching factors was used to assess differences in pathologic characteristics.

Results: 243 true interval invasive cancers were identified. Compared to 485 age matched screen-detected cancers, interval cancers were significantly more likely to be high grade (grade 3 vs. 1) (OR=4.85; 95% CI 3.00, 7.82), have lymphatic-vascular invasion (OR=4.66; 95% CI 2.97, 7.29), positive lymph nodes (OR=2.30; 95% CI 1.64, 3.21), and have a triple negative phenotype (OR=1.99; 95% CI 1.03, 3.85).

Conclusions: Controlling for age and screening interval, true interval invasive breast cancers have significantly more unfavorable prognostic variables compared to screen-detected cancers suggesting a true difference in tumor biology.

276 Breast Cancer Incidence: A Possible Relationship to Folic Acid Fortification?

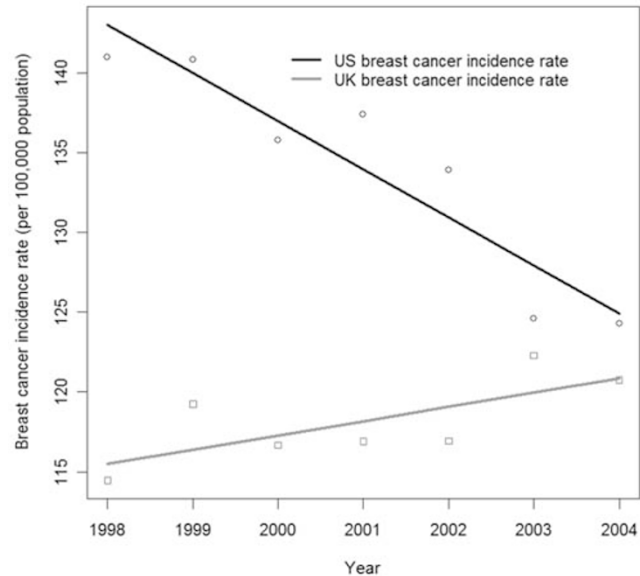*P Reddy, M Hogarth, C-S Li, JW Miller, R Green.* University of California, Davis, Sacramento, CA.

Background: In 1998 the U.S. food supply was fortified with folic acid. This was successful in reducing both the incidence of neural tube defects and the prevalence of folate deficiency. Fortification has, however, led to excess folate consumption by a significant percentage of the population. Regarding cancer, excess folate may prevent tumor initiation by maintenance of DNA integrity. Conversely, folate may promote progression of established cancers by providing a nutrient that is rate limiting for proliferating cell clones. Folate plays a key role in methylation and may influence cancer development and progression through epigenetic mechanisms. We studied effects of folic acid fortification by comparing breast cancer incidence rates in the U.S. before and after folic acid fortification with the rates in the U.K., there is no folic acid fortification.

Design: Breast cancer incidence trends from 1985-2004 were analyzed. U.S. data for breast cancer incidence was derived from the National Cancer Institute's Surveillance Epidemiology and End Results (SEER) registry. The U.K data for breast cancer incidence was derived directly from Cancer Research UK.

Results: There was a statistically significant increase in breast cancer incidence in both the U.S. and U.K. over time during the period 1985 – 1998.

After 1998, breast cancer incidence decreased significantly in the U.S. compared with the UK, where breast cancer incidence has been increasing, though not significantly.

The decline in the U.S. coincides temporally with the introduction of folic acid fortification.

Conclusions: The epidemiological evidence of decreasing breast cancer incidence in the U.S. is remarkable. Temporal relationships are consistent with a possible influence of folic acid fortification on breast cancer incidence in the U.S.

277 Genomic Profiling of Mitochondria-Rich Breast Cancer

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Background: Breast carcinomas entirely composed of cells with abundant eosinophilic cytoplasm fall into four main groups: apocrine, neuroendocrine, acinic and oncocytic carcinomas. Oncocytic carcinomas are composed of mitochondria-rich cells (mitochondria-rich breast cancer: mt-rich BC) and their status as a discrete pathological entity remains a matter of contention. Our aim was to define the molecular genetic features of mt-rich BCs using high-resolution microarray-based comparative genomic hybridisation (aCGH) and to compare these profiles with those of a series of grade and oestrogen receptor (ER) status-matched invasive ductal carcinomas of no special type (IDC-NST).

Design: Eighteen mt-rich BCs were retrieved from the files of the Department of Pathology at Bellaria Hospital, University of Bologna. Cases were graded according to Elston & Ellis and ER was assessed using the Ventana system. Eighteen mt-rich BCs and a series of 36 grade and ER matched IDC-NSTs were microdissected and subjected to aCGH using a 32K tiling path bacterial artificial chromosome array platform. aCGH results were subjected to unsupervised hierarchical clustering analysis. Profiles were compared using a previously validated multi-Fisher's exact test with p values adjusted for multiple comparison by the false discovery rate.

Results: Unsupervised analysis demonstrated that mt-rich BC preferentially formed a distinct cluster. Multi-Fisher's exact test revealed that mt-rich BC significantly differed from IDC-NSTs at the genomic level. Gains on chromosomes 3q29, 5q35.2, 6p21.31, 7q22.1, 8p11.1, 9q34.11, 11q13.1-q13.2, 12q13.13, 16p13.3, 17q21.2, 19q13.12-19q13.2 and 20q11.21, and losses on 2p11.2, 3p12.2, 4p15.32, 5q21.3, 6p22.1, 7q31.31-q31.32, 10q23.1, 11p14.1, 12q24.11, 14q13.3-q21.1, 15q15.1-q15.3, 18q21.32, 21q21.3 and Xq13.1 were more prevalent in mt-rich BC. High-level gains/amplifications of chromosome 5q23.2, 6q24.2, 7q34, 8q23.1-q23.2, 9q34.3 and 17q25.3 were significantly associated with mt-rich BC.

Conclusions: Our study provides the first high-resolution molecular genetic analysis of mt-rich BCs, which revealed that these tumours are heterogeneous at the genetic level. Mt-rich BCs have distinct histological features and molecular genetic profiles supporting the contention that they may constitute a distinct pathological entity.
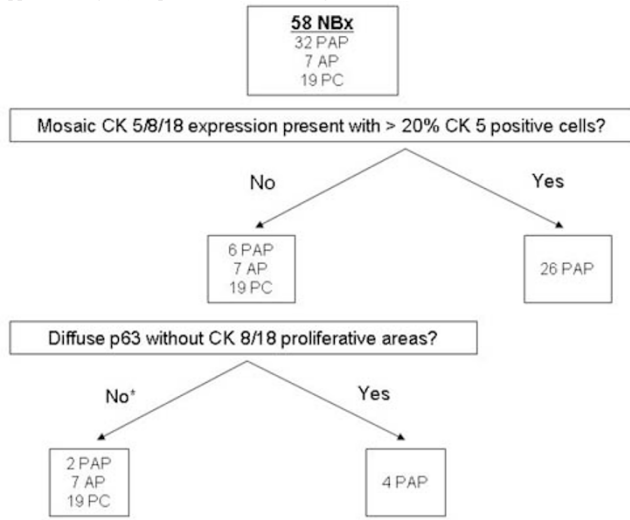
278 Evaluation of the Predictive Ability of a CK5/p63/CK8/18 Antibody Cocktail in Stratifying Breast Papillary Lesions (BPLs) on Needle Biopsy (NBx): An Algorithmic Approach Works Best

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Background: We have previously found that breast papillomas (PAPs), atypical papillomas (APs) and (PCs) have different expression profiles as seen with a CK5/p63/CK8/18 antibody cocktail. The aim of this follow up study is to compare the utility of various components of this antibody cocktail in the evaluation of BPLs in NBx material.

Design: A series of 58 BPLs in NBx specimens were immunostained with a CK5/p63/CK8/18 antibody cocktail and evaluated without knowledge of the primary or the available follow up excisional biopsy (EBx) diagnosis (Dx) (32 PAPs, 7 APs and 19 PCs). Based on cutoffs obtained from our prior study, cases were characterized based on the presence or absence of a diffuse mosaic CK5/CK8/18 expression pattern with at least 20% CK5 positive cells, and on the presence or absence of at least a focal proliferative area with 80% or more CK8/18 positive cells. p63 expression was categorized as negative, diffuse (uniformly distributed staining in at least 1 myoepithelial cell per

10 non-peripheral epithelial cells), or intermediate (remainder). Using EBx Dx as the gold standard, we compared the sensitivity (Sn), specificity (Sp), positive (PPV) and negative predictive value (NPV) of the different markers, including an algorithmic approach (Figure) to predict a non-PAP diagnosis on EBx.

* Includes cases without diffuse p63 and/or at least a focal CK 8/18 proliferative area

Results:

	Sn(%) [95% CI*]	Sp(%) [95%CI]	PPV(%) [95%CI]	NPV(%) [95%CI]
Original Dx	81 [60-93]	91 [74-98]	88 [67-97]	85 [68-94]
CK8/18 alone	100 [84-100]	81 [63-92]	81 [63-92]	100 [84-100]
p63 alone	96 [78-100]	59 [41-76]	66 [49-80]	95 [73-100]
Algorithmic	100 [84-100]	94 [78-99]	93 [75-99]	100 [86-100]

*CI, confidence interval

Conclusions: Compared to histology alone, differential CK5/8/18 expression, p63 expression, or an algorithmic approach using these markers in combination all have a higher Sn for a non-PAP diagnosis on EBx; however the algorithmic approach had the highest Sp and a 100% NPV. Use of a CK5/p63/CK8/18 antibody cocktail allows for more definitive classification of BPLs on NBx with the potential for more limited EBx procedures.

279 Mammaglobin – Based Assays for Detection of Minimal Residual Mammary Cancer Will Miss All Metaplastic and Medullary Carcinomas
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Background: Mammaglobin (MGB) and Cytokeratin 19 (CK19) are the two most commonly utilized markers for the detection of minimal residual mammary carcinomas in sentinel lymph nodes or bone marrow. The purpose of this study was to explore the expression of these markers in common histologic subtypes of breast cancer.
Design: Core needle, or excisional biopsies from 1,123 untreated invasive mammary carcinomas were evaluated for the immunohistochemical expression of MGB and CK19. The staining result for MGB was semiquantitated as 1+ (less than 10% positive cells), 2+ (11% to 25% positive cells) and 3+ (more than 26% positive cells).
Results: Thirty-six carcinomas were classified as metaplastic (based on morphology; triple-negative, p63+), 38 as medullary (based on morphology; triple-negative; HLA-DR+), 44 lobular (morphology; E-Cadherin -) and 1,005 ductal carcinomas. All metaplastic and medullary carcinomas were negative for MGB. Thirty-three lobular (75%) and 492 ductal carcinomas (49%) showed positive reaction for MGB (1+= 43%; 2+= 23%; 3+= 34%). CK19 was expressed by 97% of all mammary carcinomas.
Conclusions: Mammaglobin-based assays for detection of minimal residual disease will fail to detect all medullary and metaplastic mammary cancers as well as more than 50% of ductal carcinomas.

280 Novel Non-Biotin Polymeric Immunohistochemical Visualization Systems Improve Estrogen Receptor Evaluation in Breast Cancer
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Background: A novel generation of immunohistochemical (IHC) visualization systems based on a non-biotin polymeric (NBP) technology has been released recently. We compared the new NBP and the streptavidin-biotin systems (SAB) to evaluate estrogen receptor (ER) in breast carcinomas.
Design: Serial sections from a tissue microarray (TMA) containing 320 invasive breast carcinomas were marked for ER using the rabbit monoclonal antibody SP1. Eleven different visualization systems were used, including 7 NBP and 4 SAB following the instructions provided by the suppliers. All slides were scanned using Mirax Scan, Zeiss™, and the intensity of IHC staining was automated quantified using HistoQuant™ software. The background was visually evaluated as absent (0), weak (1), moderate (2), or strong (3).
Results: NBP Advance™ and Novolink™, and the SAB LSAB+ showed the strongest staining intensity (P<0.01). Advance™ also showed 2 positive cases that were considered

negative when manually evaluated with all the other systems. The seven NBP showed no background and sharper staining when compared to SAB systems (p<0.05). NBP PicTureMAX, showed the least background and SAB LSAB+ showed the most.

Comparison of eleven different visualization systems grouped according their staining intensity level expressed by the p value

Staining intensity level	Non-biotin polymer system (p value*)	Streptavidin-biotin polymer (p value*)
Stronger	Advance (0.0034) / NovoLink (0.0061)	LSAB+ (0.03)
Up intermediate	Super Sensitive non-biotin HRP (0.01) / PicTure Max (0.01)	Super Sensitive (0.02)
Low intermediate	Super PicTure (0.01) / Mouse or rabbit Polydetector (0.01)	Mouse or rabbit Immunodetector / EasyPath
Weaker	EnVision+	----------

* p value of the statistical analysis between the staining intensity of each visualization system and the group of systems from the level below.
Conclusions: The NBP provide sharper IHC signal without background. The NBP Advance™ and Novolink™, followed by all the other 2nd generation NBP, represent a powerful tool for reliable standardization of IHC for ER evaluation in breast carcinomas.

281 HER2 Status Determined on Pre Operative Biopsies and in Operative Specimens: Concordance among Immunohistochemistry (IHC) Fluorescence *In Situ* Hybridization (FISH) and Chromogenic *In Situ* Hybridization (CISH) Techniques in the CRITHER Study
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Background: In breast cancer, HER2 status is assessed by IHC or FISH on operative tissue sample and *In situ* hybridization techniques are standard tools for determining equivocal IHC 2+ overexpression and deciding the correct treatment for HER2-positive breast cancer patients. Oncologists sometimes ask for assessment of HER2 status on per cutaneous core biopsy (CB).The objective of the CRITHER study was to investigate concordance between the results of CISH, FISH, and IHC techniques on pre-operative CB samples for HER2 status compared with status determined by FISH on surgical specimen from untreated non-metastatic breast cancer patients in France.
Design: Analysis was performed on samples from 260 patients recruited at 24 pathology centers for the period 2003 to 2006 inclusive. Recruitment was made on the basis of IHC obtained from operative tissue samples in order to obtain around 50% HER2-positive (3+), 30% HER2-negative (0/1+), and 20% HER2-equivocal (2+) cases. CISH and IHC were performed on the pre-operative CB and matched surgical specimen in the participating laboratories, and FISH on both specimens in each of 4 reference pathology centers. IHC staining was analyzed according to different scores.
Results: The rate of discordance between pre-operative CISH and operative FISH was 1.8% with 1.5% false-positives and 2.2% false-negatives (k=0.963; p<0.001); the corresponding rate for pre-operative IHC (excluding IHC 2+ cases) and operative FISH was 1.5% with 1.6% false-positives and 1.4% false-negatives (k=0.953; p<0.001). Comparison of pre-operative and operative samples showed expected low rates of discordance for CISH (0.85%; k=0.982; p<0.001) and FISH (0.45%; k=0.991; p<0.001), with no false positives. Results for the IHC scoring system analyses on concordance will be presented at the meeting.
Conclusions: Concordance for both CISH and FISH between pre-operative and operative results was excellent. There was a high rate of concordance for pre-operative CISH and IHC HER2 status determination on CB (excluding IHC 2+ cases) compared with operative FISH results in patients with previously untreated non-metastatic breast cancer.

282 Polycomb Transcription Factor Bmi-1 Expression Is Associated with Favorable Outcome in Mammary Carcinoma
JS Ross, E Slodkowska, CE Sheehan. Albany Medical College, Albany, NY.
Background: The polycomb transcription factor Bmi-1 originally linked to lymphomagenesis has recently been associated with tumor cell renewal and the stem cell phenotype in experimental breast cancer models. Bmi-1 appears to function as a tumor suppressor by up-regulating the INK4A locus and inducing growth arrest, cellular senescence and apoptosis. The clinical significance of Bmi-1 expression in breast cancer has not been fully examined.
Design: Formalin-fixed, paraffin-embedded tissue sections from 180 cases of invasive mammary carcinoma (130 ductal carcinomas (IDC) and 50 lobular carcinomas (ILC) were immunostained by automated methods (Ventana Medical Systems Inc., Tucson, AZ) using mouse monoclonal Bmi-1 antibody (Upstate/Millipore, Temecula, CA). Tumor immunoreactivity was semiquantitatively scored based on staining intensity (weak, moderate, intense) and percentage of positive cells (focal <= 10%, regional 11-50%, diffuse >50%) in all cases.
Results: Bmi-1 immunoreactivity was a predominantly nuclear pattern in the majority of cases. Nuclear Bmi-1 positivity correlated with tumor type [36/50 (72%) ILC vs 69/130 (53%) IDC, p= 0.02], low tumor grade [67% grade 1 vs 58% grade 2 vs 36% grade 3, p=0.02], low stage [64% low stage vs 45% advanced stage, p=0.05], ER status [74% ER positive vs 31% ER negative, p<0.0001], PR status [70% PR positive vs 42% PR negative, p<0.0001], and lack of disease recurrence [68% non-recurrent vs 39% recurrent, p<0.0001]. Within the ILC subgroup, nuclear Bmi-1 nuclear immunoreactivity correlated with patient survival [95% alive vs 60% expired, p=0.007]. Within the IDC subgroup, nuclear positivity showed a trend toward correlation with a later age at diagnosis [36% <45 years of age vs 48% diagnosed between 45-55 years of age vs 61% >55 years, p=0.067].
Conclusions: Nuclear Bmi-1 expression in invasive mammary carcinoma is a frequent

event and correlates with favorable prognostic factors including advanced patient age, lobular differentiation, low tumor grade, early tumor stage, positive ER and PR status, disease recurrence-free survival (IDC and ILC groups) and overall survival (ILC group only). Further study of Bmi-1 expression in mammary carcinoma appears warranted.

283 *p53* Mutation Correlates with Molecular Profile and Outperforms p53 Immunohistochemistry (IHC) in Predicting Response to Neo-Adjuvant Chemotherapy in Operable Early Stage Breast Cancer (ESBC)

JS Ross, CM Perou, E Slodkowska, MS Ross, AB Boguniewicz, EF McKenna, HJ Lawrence, M Royce, S Gluck. Albany Medical College, Albany, NY; U North Carolina, Chapel Hill, NC; XeNa Trialists and Roche Molecular Systems, Pleasanton, CA; U New Mexico, Albuquerque, NM; Miami U, Miami, FL.

Background: The XeNa Phase II muticenter trial of neoadjuvant capecitabine, docetaxel +/- trastuzumab enrolled 157 patients with ESBC (T2-T3, N0-N1). The primary endpoint was the rate of pathologic complete response (pCR) and near-complete response (npCR). A secondary endpoint was to evaluate the association between *p53* alterations with standard biomarkers, molecular profiling and response to chemotherapy.

Design: *p53* mutation status was determined by a microarray-based resequencing assay (ACHIP) (AmpliChip p53 – Roche Molecular Systems, Pleasanton, CA); and by standard IHC staining for p53 protein using the Bp-53-11 antibody (Ventana, Tucson, AZ). Gene expression profiling for breast cancer subtypes was performed using a new 50-gene centroid-based method (PAM50).

Results: 78/157 (50%) of cases had *p53* mutations (70% missense, 17% nonsense, 11% frame shift and 1% splice site). In 78/120 (65%) cases IHC was concordant with ACHIP. IHC was negative in 16/60 samples (27%) which had ACHIP mutations with 10/16 (63%) featuring non-missense mutations likely to result in no detectable p53 protein. IHC was positive in 26/60 cases (43%) which were ACHIP wild type. *P53* mutations were highest in Basal-like (75%) and HER2-enriched (57%) molecular subtypes. P53 IHC varied little with molecular subtypes (35 to 50%) and did not correlate with ACHIP status. The rate of pCR and npCR was significantly higher in cases with ACHIP *p53* mutations (20/58 - 35%) than in cases without mutations (7/52 - 13%; p = 0.014; 2-tailed Fisher's Exact Test), and this was apparent in both HER2$^+$ and HER2$^-$ cases. IHC results did not predict treatment response (18% pCR and npCR in IHC-negative cases vs. 27% in IHC-positive cases; p = 0.35). PAM50 expression subtype (p<0.001), ER- (p=0.0001) and HER2+ (p=0.0004) status also predicted for pCR and npCR.

Conclusions: *P53* mutations are frequent in Basal-like and HER2-enriched subtypes and predict pCR and npCR for this regimen. IHC lacks sensitivity and specificity for *p53* mutations and does not predict therapy response. In addition to ER-/HER2+ status, the new PAM50 expression profiling subtypes correlated with *p53* mutation status and also predicted therapy response.

284 Columnar Cell Lesions Diagnosed by Core Needle Biopsy of the Breast: Correlation with Surgical Excision

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Background: Columnar cell lesions (CCLs) of the breast have been gaining attention as potential nonobligate precursor or even an earliest recognizable form of low-grade intraductal and some invasive carcinomas. However, there is no consensus on management of these lesions diagnosed on percutaneous imaging-guided core needle biopsy (CNB).

Design: We have retrospectively reviewed CNBs with less than malignant diagnosis dating from 1997 to 2008 which had a subsequent excision. This review identified 59 CNBs with CCLs in 57 women (age 34-85, average 56.3). We used the morphologic schema by Schnitt to classify these lesions into columnar cell change (CCC), columnar cell hyperplasia (CCH), and flat epithelial atypia (FEA) which comprises CCC and CCH with atypia. 47 (80%) lesions presented with microcalcifications and were biopsied under stereotactic guidance using 11g vacuum-assisted probe; 12 (20%) presented as a mass and were biopsied under ultrasound guidance with a 14g needle.

Results: 11 of 59 (19%) cases were upgraded to malignant on excision, including one invasive mucinous carcinoma presenting as a mass; the remainder were low-grade DCIS.

Feature on CNB	Malignancy on Excision/Total Number of Cases	p
microcalcifications	5/47 (11%)	0.002
mass	6/12 (50%)	
ducts with CCL	.	
1-3	3/17 (18%)	
4-7	3/23 (13%)	NS
8-10	2/10 (20%)	
>10	1/9 (11%)	
CCC/CCH	2/14 (14%), 1 invasive mucinous	NS
FEA	4/16 (25%)	0.04
CCL with ADH	4/26 (15%)	NS
FEA with ADH	3/23 (13%)	NS
CCL with mucocele	0/11 (0%)	NS
CCL with lobular neoplasia	1/6 (16%)	NS
CCL with radial scar	0/3 (0%)	NS
TOTAL	11/59 (19%)	

Conclusions: To the best of our knowledge, this is the largest study of surgical follow-up of CCLs diagnosed on CNB. The rate of upgrade to malignancy was slightly higher for FEA than for non-atypical CCL. ADH, lobular neoplasia and mucocele did not increase the likelihood of upgrade. The only features predictive of malignancy on excision were presentation as a mass and FEA. Excision should be recommended for FEA with or without ADH or lobular neoplasia. Consideration should be given to excision of masses showing any CCL on CNB.

285 Axilary Lymph Node Metastatsis in Primary Osteogenic Sarcomas of the Breast

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Background: Pure mammary osteogenic sarcomas are rare, representing a fraction of 1% of primary breast malignancies. Regional lymph node metastasis has rarely been documented in primary breast osteosarcomas. Against this background, a small series is presented that includes one case with axillary lymph node metastasis. Features of this case that may explain the nodal metastasis are discussed.

Design: The surgical pathology files of our institution from 1988 to 2008 were searched and the cases of osteogenic sarcoma were retrieved. Pathology material were reviewed and clinical information including MRI, mammographic findings, and clinical follow up were obtained. The possibility of metaplastic carcinoma was excluded by using appropriate immunostains. None of the tumors had a phylloides component.

Results: All patients were women who ranged in age from 32-85 years (mean= 58). Six tumors were in the left and one in the right side. Initial treatment ranged from incisional biopsy (n=2) to mastectomy (n=5); one of the former had subsequent completion mastectomy. Tumor size ranged from 2.8 to 17 cm (mean= 7.3 cm). Mitotic figures ranged from 20-60/ 10 HPF. Five cases had marked cytologic atypia. Five cases were of osteoblastic and 2 of osteoclastic type. The 17 cm tumor with ulceration through the areolar region of the breast had metastatic disease in one of ten axillary lymph nodes. There was no direct extension to the axillary node by the tumor. Follow up was available in 4 cases: bone metastasis developed within 3 years in one case, one patient died of disease within a month of surgery due to complications of surgery (the case with axillary lymph node metastasis), and two patients are alive with no evidence of disease 2 years post diagnosis.

Conclusions: The presence of axillary lymph node metastasis in one of the 7 tumors in our series is a most unusual finding given the rarity of lymph node involvement associated with sarcomas in general and osteogenic sarcomas in particular. It is postulated that involvement of the areolar skin, where a rich lymphatic network exists, is the major factor responsible for lymph node metastasis.

286 Assessment of Prognostic Value of Classical Clinical-Pathological Factors in Hormone Receptor Positive Breast Carcinoma

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Background: The prognosis of patients with estrogen receptor (ER) and/or progesterone receptor (PgR) positive breast carcinoma (BC) can be highly variable. The aim of this study was to investigate the clinical-pathologic features with potential prognostic significance such as age, tumor size, histologic grade, Bcl2, Ki67, Her2, p53 and immunophenotype in a series of BC hormone receptor (HR) positive.

Design: A total of 511 HR-positive BCs with axillary dissection and without neoadjuvant treatment were retrieved from the Surgical Pathology files. Median clinical follow-up of the patients was 78 months (range 15 to 245 months). Age ranged from 20 to 88 years (median 56 months). Histologic grade (HG) was assessed according to the Nottingham criteria. Immunohistochemical staining was performed for Bcl2 (cut-of 50%), ER (cut-of 10%), PgR (cut-off 10%), Ki67 (cut-off 20%), p53 (cut-off 20%) and Her2 (2+ and <30% 3+ confirmed by FISH). HR-positive/Her2-negative tumors were classified as luminal "low-risk" (Ki67/p53 <20%) and luminal "high-risk" (Ki67/p53 ≥20%). Significant associations were identified using Chi-square and Fisher's exact test. Actuarial survival was calculated by the Kaplan-Meier method (log rank test) and multivariate Cox analysis was applied. A p-value <0.05 was considered significant.

Results: Tumors were predominantly of ductal type (93 %), <20 mm in size (57%) and negative lymph nodes (64 %). HG was G1 in 23%, G2 in 43% and G3 in 33%. Increased Ki67 was observed in 27% of tumors, Her-2-positive in 15%, p53 in 13% and low Bcl2 in 27%. High HG correlated with high Ki67, p53 positive, low Bcl2, Her2-positive and luminal "high risk" phenotypes (all p<0.05). Poor survival was seen for patients with larger tumors, of G3, positive lymph nodes, high expression of Ki67, low Bcl2, Her2-positive and "high risk" phenotypes (all p<0.02). However, a multivariate analysis revealed that only lymph node status, tumor size and HG were significant independent predictors of survival (all p<0.05).

Conclusions: Our findings in a series of BC with positive HR status support the usefulness of the immunophenotype classification. Nevertheless, classical pathological features such as HG, lymph node status and tumor size are the most powerful independent prognostic factors. Supported by grant FIS PI061488.

287 MammaPrint Predicts Survival in Small Breast Cancer Tumors

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Background: A 70-gene tumor expression profile was established as a powerful predictor of disease outcome in breast cancer patients. The test known as "MammaPrint" was recently validated in independent cohorts and implementation was shown to be feasible in community hospitals. According to most clinical guidelines, small size breast cancer are identified to be at lower risk for developing distant metastasis. In this study, we investigate the performance of MammaPrint in patients with small tumors.

Design: 13 patients with small tumors (< 2cm) treated at the Hospital of Bamberg were examined by traditional clinicopathological factors (angioinvasion and immunohistochemical investigation for estrogen, progesterone and Her-2 receptor) and risk assessment was compared to MammaPrint results. To assess the accuracy of MammaPrint risk classification in this patient group, we determined MammaPrint results and outcome in patients with tumors smaller than 1 and 2 cm using 319 samples derived form publicly available data sets.

Results: The analysis of the 13 tumors showed that 2 tumors belonged to the low-risk-

group, 6 to the intermediate and 5 to the high-risk-group according to the St Gallen criteria. MammaPrint confirmed the clinical low- and high-risk-cases and classified 3 of the intermediate tumors as low-risk and the other 3 as high-risk-cases. In the retrospective analysis, Kaplan Meier analysis of the 319 tumors showed a significant separation in the probability of developing distant metastases at 10 years according to MammaPrint outcome. In the 280 patients with tumors between 11 and 20 mm, the probability of remaining free of distant metastasis at 10 years was 85% in the group with good prognosis signature (44% of patients) and 60% in the group of patients with the poor prognosis signature. Moreover, even in the very small tumors (< 1cm), a high risk group with poor outcome could be identified.

Conclusions: MammaPrint provides more accurate information on recurrence risk in small tumors as compared to conventional clinical criteria and will improve the guidance for the requirement of adjuvant therapy for woman diagnosed with breast cancer. Especially, MammaPrint accurately classifies tumors with intermediate risk for progress into low- and high-risk-cases.

288 Benign Breast Disease in African-American Women
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Background: Women with benign breast disease (BBD) represent a large and clinically important population. Specific histologic findings in BBD have been shown to be strong indicators of later risk of breast cancer. The major studies of BBD performed to date have been based primarily in Caucasian-American (CA) women. Thus, the prevalence and distribution pattern of BBD in African American (AA) women is not well known.
Design: We reviewed archival H and E stained sections of breast needle core and excisional biopsies performed on all AA women in the years 1998 to 2000 at our institution and diagnosed with BBD. BBD was classified, by one pathologist, as nonproliferative (NP), proliferative disease without atypia (PDWA) or atypical hyperplasia including ductal and lobular types (AH), using standard microscopic criteria. We also examined the status of lobular involution in the same biospies and classified it as none or absent (<1%), partial (1-75%), or complete (75%). We compared lobular involution in our population to that of a cohort of CA women with a diagnosis of BBD within the same age category (<45y; 45-55y and >55y).
Results: We identified 520 AA patients with a diagnosis of BBD on breast biopsy. The mean age at diagnosis was 46.4 years (14.7 y). Seventy-five percent were diagnosed with NP, 22% with PDWA, and 3% with AH. Lobular involution increased with age. In women older than 55 years however, the increase in lobular involution appeared to be slower in AA than in CA women (CA had 2.7% none vs. 44% complete and AA had 19% none vs. 31% complete; p<0.001). There was no difference between AA and CA women younger than 55 years in regard to the presence of lobular involution. Lobular involution was similar throughout the different BBD categories in the AA population.
Conclusions: In our series of AA women with BBD, the distribution of BBD appears to be similar to other series (including mainly CA women). Our data on lobular involution may indicate that AA undergo lobular involution at a different rate than CA women.

289 CSF-1 and Fibromatosis Expression in Stroma of Ductal Carcinoma In Situ
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Background: Recent advances in the study of the tumor microenvironment (TME) have revealed the importance of the interaction between the tumor cells and their surrounding stroma in various malignancies. Our lab has previously shown that the CSF-1 and DTF signatures define two stromal reaction patterns seen in the microenvironment of invasive breast cancer (West Lab Invest, 2008; 88: 591). In this study we determine whether these signatures are also present in ductal carcinoma in situ (DCIS).
Design: Four markers for the CSF-1 response (CD163, FCGR3A, CTSL1, FCGR2B) and four markers for the DTF response (SPARC, MMP11, CDH11, SDC1) were examined using immunohistochemistry on tissue microarray (TMA) for 230 women with DCIS. The CSF-1 and DTF response was scored as 0 or 1 (no staining or <30% of cells showing strong staining) or 2 (>30% of cells showing strong staining). Scores were summed for all four markers within each signature. A sum of 3 or more was considered positive, and a sum of 2 or less was considered negative.
Results: 44% of all cases were positive for the CSF-1 signature and 40% were positive for the DTF signature. 24% of all cases showed a positive signature for both CSF-1 and DTF. In addition, there was increased expression of both the DTF and CSF-1 markers in High grade tumors when compared to Low grade tumors. There was a 1.8 X (p = 0.0002) increase in the percentage of cases positive for the DTF markers and a 2.8X (p < 0.0001) increase in the percentage of cases positive for the CSF-1 markers in High grade compared to Low grade tumors.
Conclusions: The CSF-1 and DTF gene signatures define stromal responses in DCIS. The level of stromal expression increases as grade increases. This study demonstrates that there are differential stromal reaction patterns in DCIS and that they may be similar to those seen in invasive carcinoma.

290 Comparative and Additive Sensitivities of Immunohistochemical Markers of Breast Cancer Using New Monoclonal Antibodies to GCDFP-15 and Mammaglobin
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Background: Gross cystic disease fluid protein-15 (GCDFP-15) is a glycoprotein expressed by apocrine cells and in primary breast carcinomas. Mammaglobin is expressed almost exclusively in mammary epithelium, and is also a specific marker for

breast carcinoma. Previous studies have shown that the sensitivities of GCDFP-15 and mammaglobin in breast carcinomas are both around 50% with a significant increase in sensitivity when combined (~70%). Expression of both proteins has been used to identify breast carcinoma in the context of carcinoma of unknown origin. We wished to test the sensitivity of a new mouse monoclonal antibody (MoAb) to GCDFP-15 and compare its sensitivity to mammaglobin for the detection of breast carcinomas.
Design: A series of 322 breast carcinomas were tested on whole tissue sections for expression of GCDFP-15 and mammaglobin by immunohistochemistry (IHC) using the mouse MoAb to GCDFP-15 (Novocastra, clone 23A3) and a rabbit MoAb to mammaglobin (Zeta, Corp, clone 31A5). Antibody detection was by EnVision Plus™ polymer IHC (DAKO, Carpinteria, CA.) Scoring was based on percentage of positive tumor cells: negative (0%), rare cells (<1%), focal (1-25%), variable (26-75%), uniform (>75%).
Results: The overall sensitivity of the MoAb antibody to GCDFP-15 was 80.4%, compared with 59.9% for mammaglobin; 13.3% of breast cancers were negative with both antibodies, yielding a combined sensitivity of 86.7%. The number of cases that were GCDFP-15 positive/mammaglobin negative was 86/322 (26.7%) and GCDFP-15 negative/mammaglobin positive was 20/322 (6.2%.) Many of the cases showed heterogeneous staining for GCDFP-15 with 44/322 (13.7%) cases with only rare cells positive.
Conclusions: Clone 23A3, a new monoclonal antibody to GCDFP-15 demonstrated considerably higher sensitivity (80.4%) compared with the ~50% sensitivity historically reported for earlier generation anti-GCDFP-15 antibodies for the detection of breast carcinomas. There was a small subset of cases (6.2%) that showed expression of mammaglobin but were negative for GCDFP-15. Evaluation of these two antigens together may maximize the ability to identify breast carcinoma when tumor origin is unknown. The expression of GCDFP-15 was heterogeneous in many tumors, with 13.7% showing only rare cells positive (<1%.) Therefore the increase in sensitivity seen in this report may not be replicable in tissue microarray-based studies.

291 Tumor Proteomic Profiling Predicts the Resistance of Breast Cancer to Chemotherapy
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Background: Chemotherapy is widely used in breast cancer treatment, but the outcomes vary with some patients responding well and others responding poorly. We hypothesized that the profile of the differentially expressed proteins in tumor tissue may predict individual drug response.
Design: The SELDI-TOF mass spectrometric profiles of tumor tissues obtained from drug resistant and drug sensitive tumors were compared to identify the differences between the two. Fifty-two T2-T4 breast cancer tissues obtained prior to neoadjuvant chemotherapy were analyzed. Of these the first two thirds (35 cases) were allocated to a training set to select m/z peaks characteristic of resistant tumors. The candidate m/z peaks were used to develop a predicting rule to evaluate the remaining 17 specimens in the validation set.
Results: The proteomic peak differences were found most prominent between the drug-resistant breast tumors compared with those with various sensitivity by non-supervised hierarchical clustering. In the supervised classification, the KNN model with K=1 correctly classified 100% of resistant tumors (4/4), and 84.6% of the tumors with favorable response (11/13) with an accuracy rate of 92.3% in the validation set. Furthermore, a single peak at m/z 16,906 correctly separated 88.9% of the tumors with pathologically complete response, and 91.7% of the resistant tumors in the entire group.
Conclusions: The data suggests that breast cancer protein biomarker profiling may be used to pre-select patients for optimal treatment.

292 DNA Methylation of ESR1 and PGR in Breast Cancer: Relationship to Pathologic and Epidemiologic Features
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Background: DNA hypermethylation of ESR1 and PGR promoters may be related to ER or PR negative breast cancer. Most data have been derived from small case series and cell lines. Accordingly, we assessed the association between methylation of ESR1 and PGR and ER and PR expression in a population-based study.
Design: We evaluated 200 invasive breast cancers included in a population-based case-control study of 2,386 cases and 2,502 controls conducted in Poland (2000-2003). Using matched hematoxylin and eosin stained slides, 0.6 mm-diameter tissue microarray needles were used to remove tumor-rich cores from formalin fixed paraffin embedded tissues. Following methodologic validation studies, cores were used to prepare bisulfite treated DNA that was tested with MethyLight to assess methylation at 4 CpG islands in promoters: ESR1-1A; ESR1-M3B; PGR-M1A and PGR-M2B and a CpG-rich region, ESR1-M4C. Methylation results were compared to ER, PR and HER2 expression assessed by Automated Quantitative Analysis (AQUA) performed on tissue microarrays, tumor features,and breast cancer risk factors.
Results: When analyzed categorically, methylation of ESR1-M3B and –M4C were weakly associated with reduced ER and PR expression and ESR-1-M3B was positively related to HER2 (all reults non-significant). When ESR1-M4C methylation was analyzed continuously, it was significantly inversely related to ER and PR levels and positively related to HER2. In multivariate analyses including age and tumor characteristics, methylation of ESR1 was a predictor of ER, PR and HER2 status, though weaker than

tumor size. ESR1-M4C methylation was also weakly associated with younger age, pre-menopausal obesity and short duration of breast feeding.

Conclusions: Low level methylation is common in CpG-rich promoter regions of ESR1 and PGR, but relationships with protein expression are weak and generally non-significant. Strongest associations between DNA methylation and marker expression were found for ESR1-M4C, which is not a true CpG island.

293 Isolated Tumor Cells and/or Micrometastasis in Breast Sentinel Lymph Nodes Can Be the Result of Innocuous Passive Dissemination

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Background: Increased sensitivity of breast cancer cell detection with cytokeratin IHC has resulted in the detection of isolated tumor cells (ITCs) (<0.2 mm) and micrometastasis (MM) ($\geq$0.2 mm, <2mm) in a significant percentage of sentinel lymph nodes (SLNs) negative by H&E, yet the clinical significance of these findings remains unknown.

Design: We examined these questions by conducting comparative studies of patients having undergone SLN dissections with patients having undergone de novo full axillary dissections. Both the presence of tumor cells as well as their stem cell composition defined by aldehyde dehydrogenase positivity (ALDH1+) was investigated in 4 groups of 100 patients each: H&E negative SLNs, H&E positive SLNs, H&E negative axillary dissections and H&E positive axillary dissections. The distribution of the primary breast cancers in each group (histology limited to infiltrating ductal carcinomas) were age, size and biomarker matched (p=0.5). We also presumed that a full axillary dissection would, by definition, include the SLNs.

Results: In the H&E SLN negative group, cytokeratin IHC detected ITCs in 12% and MM in 6% of the cases. In the H&E axillary negative group ITCs were present in only 3% and MM in only 1% of cases. The difference in the detection of cytokeratin positive cells (either ITCs or MM) in the SLN v axillary group was highly significant (p=0.01). Both the SNL and axillary H&E positive groups exhibiting $\geq$2mm macrometastasis contained a similar percentage of ALDH1+ stem cells (5-15%) whereas the SNL and axillary H&E negative groups containing only cytokeratin positive ITCs or MM exhibited rare (0-2%) ALDH1+ immunoreactivity (p=0.01). In the former groups of SNL and axillary positive macrometastasis, the ALDH1+ percentage was always greater than that exhibited by the primary tumor (p=0.05) whereas in the latter groups of SNL and axillary ITCs or MM, the ALDH1+ percentage was less than that of the primary tumor (p=0.05).

Conclusions: Given the fact that the SLN procedure involves extensive physical manipulation of the breast following radioactive dye injection, manipulations absent in de novo axillary dissections, our finds suggest that ITCs and/or MM in SLNs can be the result of passive dissemination. Since passive dissemination, in contrast to active metastasis, would not select for stem cells responsible for clonogenic potential, the finding of decreased ALDH1+ in ITCs and MM suggest that passive metastasis in the setting of SLNs may be innocuous.

294 Intraoperative Cytologic Evaluation of Sentinel Lymph Nodes in Breast Cancer: A Community Hospital Experience

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Background: Cytological evaluation of sentinel lymph node (SN) is a rapid method of providing intraoperative consultation to the surgeon regarding staging in patients with breast cancer. A positive finding permits an immediate complete dissection, avoiding the need for a second operation on the axilla. A negative finding obviates the need for complete dissection, thus decreasing the morbidity. The purpose of this study was to assess the reliability of intraoperative cytologic examination of SN (ICE) at a busy community hospital.

Design: A retrospective review of ICE in patients with invasive breast carcinoma was conducted from 2004 to 2007 at William Beaumont Hospital, Troy, MI. SNs were identified by radioisotope and blue dye. The SNs were evaluated by a pathologist by sectioning at 2 mm, and examining scraped smears stained by hematoxylin and eosin. The ICE result was compared to final results of permanent sections. Negative SNs in routine sections were routinely evaluated by immunohistochemical stain for CK AE1/AE3.

Results: 795 patients with invasive carcinoma underwent ICE during breast procedures (biopsy/lumpectomy, mastectomy, re-excision). The average age of patients was 58.7 years, with an average tumor size of 18.3 mm. A total of 2662 SN were examined with an average of 3.3 SN submitted per patient. 275 positive SN were identified in 125 patients. Of these, 208 were detected by ICE. The overall sensitivity of detecting a positive SN was 75.6% and specificity was 100%, with positive and negative predictive values of 100% and 97.3%, respectively. The detection of macrometastasis by ICE was 87.6% compared to 2.5% for micrometastasis and isolated tumor cells. The sensitivity for detection of positive SN was similar for patients with ductal (67.6%, n=142) and lobular (70.0%, n=30) carcinoma. Axillary dissection was completed immediately in 123 of the 125 patients when ICE was reported as positive.

Conclusions: Intraoperative cytologic examination is a simple and reliable method for evaluation of sentinel lymph nodes in patients with breast cancer. Detection of a positive SN by ICE permits complete surgical staging of the axilla, thereby avoiding the psychological, physical, and financial impact on the patient of a second operation.

295 Use of the Veridex GeneSearch™ BLN Assay in Clinical Practice To Evaluate Breast Sentinel Lymph Nodes

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Background: Sentinel lymph node (SLN) analysis is considered standard of practice for breast cancer patients. New methodologies utilizing gene expression profiling for SLN analysis have been explored and recently the FDA approved a real time PCR assay, the Veridex GeneSearch™ Breast Lymph Node (BLN) Assay, for this application. In this study, we describe our experience with this test as applied in a routine clinical setting.

Design: Seventeen consecutive eligible SLNs from 9 patients were assessed. The lymph nodes were serially sectioned perpendicular to the long axis and the first and last sections submitted for molecular analysis. This sampling method differs from the manufacturer's recommended sampling of half the node. We wanted to assess a sampling method that would still allow for the evaluation of deposit size and location by routine H&E and IHC. RNA extraction and RT-PCR were performed using the GeneSearch™ BLN Assay reagents on the Cepheid SmartCycler® to generate expression data for 3 target genes (mammaglobin, cytokeratin 19, and porphobilinogen deaminase). Gene expression results were then applied against predetermined criteria to provide a qualitative result.

Results: Of the 17 SLNs, 4 were not submitted for molecular studies (3 nodes <0.8 mm, 1 node with macrometastasis (0.7 cm)). The remaining 13 SLNs were negative by H&E and one was positive by IHC for cytokeratin 19 (<0.1 mm focus). The GeneSearch™ BLN Assay of these cases revealed 12 negative and one positive result concordant with the H&E/IHC results.

Conclusions: While this new molecular assay is performed in less than 40 minutes and has intraoperative claims, we have opted to use it as part of a SLN algorithm where only portions of H&E negative SLNs are tested. This has resulted in a drastic reduction of H&E slides reviewed per SLN and has maintained our ability to assess other morphologic features of the node. This assay has been an excellent adjunct to traditional pathologic examination of breast SLNs.

296 The Multigene Assay Oncotype Dx Is Unnecessary for Low Grade Invasive Breast Carcinoma

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Background: The multigene assay Oncotype Dx uses RT-PCR to determine the expression of a panel of 21 genes in tumor tissue. The Recurrence Score (RS) is calculated from the gene expression results, ranging from 1-100. This assay is used to aid in the decision to administer adjuvant chemotherapy following a diagnosis of lymph node negative, estrogen receptor positive invasive breast carcinoma (IBC) and is the basis for selection for the TAILORx trial.

Design: Routine pathology parameters from all cases from our institution that have been submitted for Oncotype Dx testing were reviewed and correlated with the Recurrence Score (RS). Grade was assigned using the Elston and Ellis Combined Histologic Grade. Proliferation rate was determined by mitotic count/10 HPF, from the Combined Histologic Grade. Pearson's coefficient correlation (CC) was calculated for RS and Combined Histologic Grade, proliferation rate, and progesterone receptor status. RS was considered high for values greater than 31, intermediate for values 19-30, and low when less than 19. (NEJM 2004, 351:2817).

Results: Since June 2007, 23 cases have been sent for Oncotype Dx study. All were IBC of no special type . All patients were women, with an average age of 54 years. The average size of the IBC was 1.4 cm. The relationship between grade, proliferation rate and RS status is given in Table 1.

Correlation of RS with Histologic Features

RS	inter/high grade	low grade	inter/high proliferation	low proliferation
high (>30)	3	0	3	0
inter. (19-30)	4	0	2	2
low (<19)	8	8	4	12

Histologic grade and proliferation rate correlated with RS (CC=0.50 and 0.56, respectively). This correlation was lost when low grade carcinomas (all of which had low RS) were excluded (CC=0.25). Similarly correlation with proliferation rate was reduced when cases with a low proliferation rate were excluded (CC=0.16). Progesterone receptor status was strongly correlated with RS (CC=0.84). Nineteen cases were PR positive, while 4 were PR negative; 3 of these were associated with a high RS and one had an intermediate RS.

Conclusions: Low grade carcinomas and carcinomas with a low proliferative rate are associated with a low Oncotype DX RS. Although the presence of PR does not predict an elevated RS, the absence of PR is associated with an intermediate or high RS. Oncotype DX should be reserved for intermediate or high grade invasive breast carcinomas.

297 Multivariate Analysis of Immunohistochemical Data from 6925 Breast Cancer Patients: Molecular Classification Using 6 Well Known Markers

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Background: Molecular classification of breast cancer using cluster analysis of gene expression data was shown to be a good indicator of the biology of these tumors, and correlates with the histopathology, the treatment response, and the patients outcome. Therefore, it is desirable to define these clusters routinely by immunohistochemical methods.

Design: Data from 4952 patients with invasive breast cancers from a 12 year period (1995-2007) were re-analyzed. All patient data sets include information on the Ki67, bcl2, ER, PR, and p53 immunohistology, and the tumor stage. Where available, also

the lymph node status was taken into account. These data were analyzed with regard to intrinsic clustering and relationship to lymph node status. Based on these results, rules for IHC clustering were developed to yield results comparable to clustering of gene expression data.

Results: Principal component analysis (PCA) revealed the presence of three distinct clusters of breast cancers: ER/PR/bcl2 positive tumors, HER2 positive tumors, and tumors with accelerated proliferation characterized by high scores of p53 and Ki67. HER2 positive tumor could be further subdivided into a "classical" type (no p53 overexpression, no ER positivity), an "ER positive" type (ER > 2%, no p53 overexpression), and a "p53 overexpression" type (>50% p53 pos. nuclei). The metastatic behaviour of the HER2+++/ER+ cluster was identical to other ER+ tumors, and 10%, but the other types of HER2+++ tumors showed a more highly metastatic phenotype. Therefore, HER2+++/ER+ tumor were clustered in the "Luminal B" group together with other tumors having a Ki-67 score of > 30% and separated from the "Luminal A" group without these features. HER+++ tumors with ER-/PR- formed seperate cluster. It was not possible to distinguish basal- and non-basal phenotypes of triple negative tumors using this set of markers.

Conclusions: IHC clustering of breast cancer data that correlates with the biological behavior is possible and yields roughly the same numerical distribution as could be expected from molecular clustering. An expanded set of markers, including basal cytokeratins, will be needed to get a more refined clustering.

298 Evaluation of Incomplete Tumor Regression Scores for Neoadjuvant Chemotherapy of Breast Cancer and Predictive Value of Immunohistology
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Background: Neoadjuvant chemotherapy is an important modality for locally advanced breast cancers. However, because of the difficulties to evaluate partial regression, there is no agreement how to evaluate the partial tumor response.

Design: A series of 102 patients cases that received neoadjuvant chemotherapy in a clinical trial were evaluated for histological regression scores using four different systems (Miller-Payne, Sataloff, residual cancer burden [RCB] score, and Sinn). Tumours underwent assessment before and after 2 cycles of chemotherapy by core needle biopsy, and after resection of the tumor bed using breast conserving therapy or mastectomy. Regression scores were compared with each other and with predictability by immunohistology (ER,PR,HER2,bcl2,Ki67,p53) before chemotherapy.

Results: In 17 patients a complete histological tumor regression was observed (pCR according to RBC score or Miller-Payne regression grade 5), but near total or total regression was seen in 27 cases (TA according to Sataloff). Only 9 cases achieved complete regression of invasive and intraductal carcinoma (score 5 according to Sinn). Regression scores were very similar with the Miller-Payne system and RCB scores, but did not well correlate with the Sataloff scores with 57% of RCB incomplete regression (classes 1,2,3) being in Sataloff categories TA or TB (good responders). With lymph-node negative patients, 24% showed evidence of complete regressed lymph node metastases (Sataloff NA), while 61% of lymph-node positive patients had evidence of regressive changes (Sataloff NC). When compared with immunohistology before chemotherapy, predictive parameters were similar for complete and near complete remission (HER2 overexpression, high proliferative index, ER negativity).

Conclusions: With the use of a regression grading system it is possible to better describe the effect of neoadjuvant chemotherapy, but there is only limited correlation between the four most commonly used scoring systems. The most important difference is the definition of complete tumor regression. For statistical purposes the regression is best evaluated with the continuous residual cancer burden (RCB) score. The Sataloff score is offers the benefit to classify regression in lymph node metastases.

299 Pathologic Findings in MRI-Directed Needle Core Biopsies of the Breast in Patients with Newly Diagnosed Breast Cancer
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Background: For patients with newly diagnosed breast carcinoma, evaluation of the extent of the disease in the breast is of paramount importance in planning appropriate surgical therapy. Magnetic resonance imaging (MRI) plays an ever increasing role in the evaluation of additional areas in the affected breast deemed suspicious by MRI but indeterminate by other radiologic modalities. In this study we evaluated the pathologic findings in MRI-directed needle core biopsies of the breast directed against other suspicious areas in the affected breast of patients with a new diagnosis of breast carcinoma.

Design: Our study population consisted of 44 MRI-directed needle core biopsies of the breast performed on 40 patients with newly diagnosed breast carcinoma at Rush University Medical Center in Chicago (May 2007-July 2008). The histologic findings of these biopsies were reviewed and recorded.

Results: Overall 9/44 (20.4%) of our MRI-directed breast biopsies were malignant, 29/44 (66%) were benign and 6/44 (13.6%) showed atypia. Of the 9 malignant cases, 4 were infiltrating ductal carcinomas with tubular features, 2 infiltrating lobular carcinomas and 3 were ductal carcinoma in situ lesions. Of the 6 atypical cases, 2 were atypical ductal hyperplasia (ADH), 2 atypical lobular hyperplasia (ALH) and 2 showed areas of columnar cell hyperplasia with atypia. Of interest, more than one third of our benign cases (11/29, 38%) consisted of a specific complex multicystic lesion lined by apocrine metaplastic epithelium.

Conclusions: 1. MRI-directed needle core biopsies of separate lesions in the affected breast of patients with newly diagnosed breast carcinoma show additional foci of malignancy in 20% of the cases. **2.** A high percentage (66%) of these additional suspicious areas are benign by histologic examination. More specifically, MRI-directed needle core biopsies seem to target a specific complex multicystic lesion lined by apocrine metaplastic epithelium, in over one third of the cases. These findings suggest that MRI-directed core biopsies result in important change of surgical management in a significant number of cases. In addition, MRI-directed needle core biopsies often target benign lesions with specific histopathologic characteristics.

300 Human Epididymis Protein 4 (HE4) Expression Loss in Invasive Mammary Carcinoma Is Associated with Aggressive Disease and Adverse Clinical Outcome
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Background: The *WFDC2* gene encodes a WAP-type four disulphide core protein with protease activity normally localized to the epithelium of the distal epididymis known as HE4. Transcriptional profiling studies have found consistent up-regulation of HE4 in ovarian cancer and serum-based HE4 testing has recently been introduced as a novel screening method for the disease. The clinico-pathologic significance of HE4 expression in breast cancer has not been previously studied.

Design: Formalin-fixed, paraffin-embedded tissue sections from 146 cases of invasive mammary carcinoma (96 ductal carcinomas (IDC) and 50 lobular carcinomas (ILC) were immunostained by automated methods (Ventana Medical Systems Inc., Tucson, AZ) using rabbit polyclonal HE4 (Covance/Signet, Princeton, New Jersey). Tumors were similarly stained additional ER associated proteins WWP1 (Novus Biologiocals, Littleton, CO) and IGF-1R (Santa Cruz Biotechnology, Santa Cruz, CA) Cytoplasmic immunoreactivity was semiquantitatively scored based on staining intensity and distribution and the results were correlated with morphologic and prognostic variables.

Results: Cytoplasmic staining of benign breast epithelium was universally identified. Cytoplasmic HE4 overexpression was observed in 89/146 (61%) tumors. Tumor HE4 expression significantly correlated with ER positive status [67% ER positive tumors versus 49% ER negative tumors, p=0.035] and HER2 negative status [68% HER2 negative versus 36% HER2 positive, p=0.003]. HE4 expression also correlated with WWP1 expression [75% tumors with WWP1 overexpression versus 51% WWP1 not overexpressed, p=0.004] and IGF-1R [75% tumors with IGF1R overexpression versus 44% IGF1R not overexpressed, p=0.001]. Within the IDC subgroup, HE4 expression correlated with disease recurrence [73% recurrent tumors versus 49% non-recurrent, p=0.02]. On multivariate analysis, young age and advanced stage were independent predictors of recurrence.

Conclusions: HE4 is widely expressed on normal breast ductal and lobular epithelial cells. In breast cancer, expression of HE4 is associated with the ER+/WWP1+/IGF-1R+/HER2 negative subtype and predicts disease recurrence in the IDC sub-group. Further study of HE4 expression as a prognostic factor and potential target of therapy for breast cancer appears warranted.

301 Reduced Expression of Fanconi Anemia Complementation Group F (FANCF) 4 Is Associated with an Aggressive Phenotype and Shortened Survivial in Breast Cancer
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Background: The Fanconi anemia complex is composed of multiple members including the BRCA2 gene and functions to maintains DNA stability. Germline mutations in Fanconi genes are associated with cancer suceptibility. The expression of FANCF4 is regulated by promoter gene methylation and has been linked to sporadic cancer development. To date, FANCF 4 expression has not been studied as a potential prognostic factor in breast cancer.

Design: Formalin-fixed, paraffin-embedded tissue sections from 163 cases of invasive mammary carcinoma (123 ductal carcinomas (IDC) and 40 lobular carcinomas (ILC) were immunostained by automated methods (Ventana Medical Systems Inc., Tucson, AZ) using rabbit polyclonal FANCF (LifeSpan Biosciences, Seattle, WA). Cytoplasmic immunoreactivity was semiquantitatively scored based on staining intensity (weak, moderate, intense) and distribution (focal, regional, diffuse) and the results were correlated with morphologic and prognostic variables. Reduced FANCF 4 expression was defined as complete, regional or focal loss of tumor immunoreactivity compared with normal epithelial expression.

Results: For all invasive tumors, reduced cytoplasmic FANCF 4 protein expression was observed in 88/163 (54%) invasive tumors. Decreased FANCF 4 expression correlated with ER negative status [66% ER negative tumors vs 47% ER positive tumors, p=0.02], PR negative status [64% PR negative tumors vs 45% PR positive tumors, p=0.02], and shortened overall survival [60% expired vs 43% alive, p=0.03]. The association of reduced FANCF expression with advanced tumor stage reached near significance [68% advanced stage vs 50% early stage, p=0.08] for all tumors, but was significant for the ILC subgroup [90% advanced stage vs 53% early stage, p=0.03]. On multivariate analysis, young age at diagnosis, advanced stage, node positive status, HER2 positive status and disease recurrence predicted reduced overall survival.

Conclusions: Reduced FANCF 4 expression in invasive breast cancer is associated with an aggressive phenotype and shortened overall survival. Further study of the prognostic significance of FANCF 4 expression in breast cancer appears warranted.

302 COUP – TFII and Estrogen Receptor in Breast Cancer: Do They Correlate?
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Background: COUPTFII is a chicken ovalbumin promoter transcription factor. The expression of COUPTFII is reduced in antiestrogen-resistant breast cancer cell lines. Further, re-expression of COUPTFII in resistant cells increased tamoxifen-mediated inhibition of cell proliferation, decreased motility, and increased apoptosis. These support the hypothesis that COUPTFII plays a role in antiestrogen responses in sensitive

breast cancer cells and that reduced COUPTFII expression plays a role in acquired estrogen-resistance in human breast cancer. Estrogen receptor immunohistochemical staining is currently used to help determine whether breast cancer patients will benefit from antiestrogen therapy. Based on this standard, we set out to develop an immunohistochemical test for COUPTFII, to determine the levels sand patterns of expression, and to determine whether a correlation with ER expression exists.

Design: The levels of expression of COUPTFII were determined in ER negative and ER positive cell lines, in order to obtain a reference standard. Two antibodies were tested, one developed by one of the authors (CK), and the other purchased from Abcam (ab50487). A breast cancer tissue array (U.S. Biomax BR961) was stained with COUPTFII antibody (ab50487). Two independent reviewers graded the COUPTFII staining. The ER staining reported with the tissue array was used to compare with COUPTFII. A total of 86 cores, 68 malignant and 17 benign, were compared. The grading system included the intensity of staining and the percentage of cells staining (intenstity 0-3; percentage 0-100); the results were then expressed as a product of the two values (0-300). Pearson coefficient and r^2 were used to evaluate correlation between ER and COUPTFII.

Results: The ab50487 antibody showed correlation between Western blotting data and Immunohistochemistry: The ER negative cell line was negative, whereas ER positive (MCF7) was positive. Average staining for COUTFII was higher in the malignant breast cases then in benign tissue. Average staining, as expected, for ER was higher in the malignant tumors than benign breast lesions. The Pearson score was 0.14 and the Rsq was 0.019. This study showed no correlation between ER and COUPTFII staining.

Conclusions: Since the low expression of ER is correlated to antiestrogen resistance, we hypothesized that COUPTFII may show co-expression patterns with ER. After developing immunostaining for COUPTFII, we detected no such correlation neither in malignant, nor benign breast tissue in this study.

303 "Molecular Apocrine" Breast Carcinomas (MAC) Develop Brain Metastases (BM) Early with Respect to the Appearance of Systemic Metastases (SM)

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Background: BM often occur late in BC patients, and usually follow prolonged pharmacologic therapy of SM. Breast cancer (BC) classification by gene expression profiling has identified aggressive BC phenotypes. Human epidermal growth receptor-2 (HER2) positive tumors have a high incidence of BM. The phenotypes of tumors of BC patients who develop BM early relative to the appearance of SM have not been thoroughly examined. The identification of such phenotypes would allow for early detection or preventive measures and the development of selective therapies.

Design: We identified 55 cases of BC with BM from 1993-2007 which had clinical follow-up information and pathologic material available for review. All cases were evaluated for tumor type, Elston grade, and HER2 status. HER2 was tested by immunohistochemical staining (IHC), and HER2 fluorescence in-situ hybridization analysis (FISH) was conducted on all IHC 2+ cases. Estrogen receptor (ER) (49/55) were tested by IHC. All cases with > 75 % apocrine histology were tested for androgen receptor (AR) by IHC. The BC patients were divided into two groups: Group1) Those in whom SM developed prior to BM (n=24; Group 2) Those who had only BM, or whose SM developed at the time of, or after BM (n=31). Data analysis used Fisher's exact test for categorical variables.

Results: All BC were invasive; morphology was ductal not otherwise specified (IDC-NOS) (n=46), apocrine (n=7), colloid (n=1), lobular (n=1). Thirteen of the IDC-NOS cases were grade II (13/46) and 33 were grade III (33/46). All apocrine carcinomas were grade III. BC were considered MAC if they had >75% apocrine histology and were AR+/ER-. MAC represent 13% (7/55) of all our cases as compared to 1-2% of all IDC cases. All of the MAC patients were HER2+ and were in group 2 (*vide supra*) with respect to BM and SM occurrence, p=0.01. None of the SM of the 7 MAC patients occurred before their BM; 3 had only BM, while the SM of the remaining 4 patients were diagnosed at the time of or after the occurrence of BM.

Conclusions: We conclude that the incidence of MAC tumors may be increased in BC patients with BM, and that MAC patients developed BM early with respect to the occurrence of SM.

304 Indoleamine 2,3-Dioxygenase (IDO) Expression in Invasive Breast Carcinoma (IBC) Is Associated with Longer Relapse-Free Survival (RFS)

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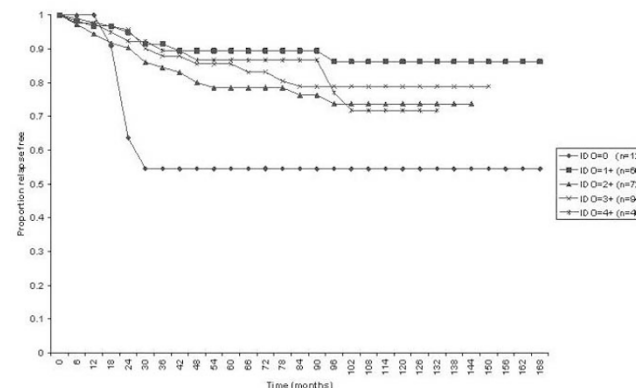
Background: Mechanisms of immune evasion are emerging as critical factors in tumor progression. The enzyme IDO catabolizes tryptophan, depleting it from the tumor microenvironment, thus preventing T-cell activation, and facilitating immune evasion. IDO is overexpressed in many human cancers, sometimes associated with poor prognosis. We report results of a tissue microarray (TMA) analysis of IDO expression in IBC.

Design: Clinical findings, follow up data (obtained from the tumor registry), and pathological data (from pathology reports and slide review) for 439 patients with IBC diagnosed between 1992 and 1998 were reviewed. Formalin-fixed paraffin-embedded tissues were plated to a TMA. The TMA was stained by IHC for ER, PR, HER2, Ki-67 as well as for IDO. The latter was semi-quantitatively scored on a 0-4+ scale and compared with clinical data and expression of markers listed above.

Results: IDO was expressed in 273 (95.8%) of 285 IBCs at 1+ or above. Preliminary analysis showed that IDO expression did not correlate with age, overall survival, tumor size, grade, nodal status, lymphocyte infiltration, or expression of ER, PR, HER2, or Ki-67. Surprisingly, IDO expression correlated with longer RFS (r=0.269; p=0.047). Kaplan Meier curves indicated this may be due to IDO-negative tumors having greater rates of early relapse (see figure). Indeed, IDO-negative tumors had shorter RFS than

IDO-positive tumors (5.0 vs 27.6 months; p=0.001) despite that IDO-positive tumors had more positive lymph nodes than IDO-negative tumors (4.3 vs 1.0; p=0.002). When other cutoffs were considered as IDO-negative (0, 1+) or positive (2+, 3+, 4+), IDO expression did not predict ER or PR expression, but IDO-positive tumors were more frequently HER2-positive than IDO-negative tumors (9.8% vs. 1.7%; p=0.048) and more likely to have a pure lobular phenotype (12.2% vs 2.8%, respectively; p=0.021).

Kaplan Meier Curves (Relapse-free Survival)

Conclusions: IDO is widely expressed in IBCs and is associated with longer RFS, HER2 expression, and lobular phenotype. Further study is necessary to replicate these findings and elucidate their significance.

305 Micrometastases in Sentinel Lymph Nodes in Breast Cancer: Clinical Implications

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Background: It remains unclear whether micrometastatic sentinel lymph node (SLN) metastases represents an adverse prognostic factor in breast cancer. Some studies have demonstrated that SLN micrometastasis has an adverse effect, whereas other studies have shown no effect on survival.

Design: All cases with SLN micrometastases were collected from our pathology files from 2003 to 2008. Small emboli of tumor cells in the lymph node sinuses and/or metastatic deposits in the lymph node parenchyma which were 0.2 mm to 2.0 mm in size were classified as micrometastases. other data collected were as follows: age, size, type and grade of primary cancer, size of SLN micrometastases, axillary node and follow-up status. Oneway Anova and Student's t tests were used for statistical analysis.

Results: There were 17 SLN micrometastases in a total of 702 SLN biopsies performed during 2003 to 2008 (2.4 %). Age of the patients ranged from 40 to 71 years (mean 53 years). 11 tumors were infiltrating ductal, 2 lobular, 2 mixed ductal and lobular, and 2 ductal carcinoma in-situ (DCIS) - 1 micropapillary and 1 comedo DCIS. Six tumors were grade 1, 6 were grade II, and 3 were grade III invasive cancers. Both DCIS showed nuclear grade III. Tumor size ranged from 0.4 to 3.9 cm (mean 1.6 cm). Lymphovascular invasion (LVI) was present in 3 of 17 cases (18%). Size of SLN metastases ranged from 0.2 mm to 2.0 mm, with multiple foci present in 1 infilating carcinoma and in 1 DCIS (12%). Axillary dissection was performed in 14 of 17 cases. Lymph node number ranged from 7 to 30. 4 cases showed additional node micrometastases (29%) and 2 cases with additional node macrometastases (14%). Follow-up was available for 14 patients and period ranged from 4 to 60 months. All patients were alive and well. There is a statistically significant correlation between tumor size and the presence of micrometastases (p<0.05). The micrometastases did not predict additional node metastases (p>0.20).

Conclusions: The size of primary tumor is significant in predicting SLN micrometastases in this study. Age of the patient, tumor type, grade, LVI, and size and number of foci of SLN micrometastases are not predictive of micrometastases or subsequent axillary node metastases. Our results have shown that SLN micrometastases have no adverse effect on patient survival. A multi-institutional study involving a large number of patients is needed to plan appropriate management of patients with SLN micrometastases.

306 Bcl-2 Is Infrequently Upregulated in Metastatic Breast Carcinomas

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Background: Bcl2 is an antiapoptotic protein which promotes cell survival. Bcl2 is expressed in normal breast epithelium, and in breast cancer its expression correlates with estrogen receptor (ER) positivity. *In vitro* data suggests that Bcl2 promotes chemoresistance (J Surg Res 1998; 76:22-26 and Endocr Relat Cancer 2000; 7:237-269) and is upregulated in metastatic breast carcinoma (MBC) (Clin Exp Metastasis 2005; 22:59-67). A direct and comprehensive comparison of Bcl2 expression between multiple distant metastases and their paired primary breast carcinomas (PBCs) has not been performed.

Design: We performed rapid autopsies (postmortem interval, 1-4 hours) on 15 consenting patients with MBC. Single-patient tissue microarrays (TMAs) were constructed from the patient's archived PBC and multiple different MBCs harvested at autopsy. TMAs were labeled by immunohistochemistry (IHC) for ER, progesterone receptor (PR), HER-2, and CK5/6 to determine the PBCs' IHC surrogate profile corresponding to breast cancer subcategories defined by gene expression profiling. TMAs were labeled for Bcl2 and expression in PBC (145 spots from 15 cases) and matched MBC (778 spots from 180 different MBCs) were compared.

Results: There were 8 ER+, HER-2- (Luminal A) cases; 5 ER-, PR-, HER-2-, CK5/6+ (Basal-like) cases; 1 ER+, HER-2+ (Luminal B) case; and 1 ER-, PR-, and HER-2+ (HER-2) case. All 9 ER+ cases (8 Luminal A, 1 Luminal B) showed Bcl2 expression in the PBC, while none of the 6 ER- cases (5 Basal-like, 1 HER-2) did (p<0.001). Four Luminal A cases lost expression of ER and/or PR in their MBC; 2 of these demonstrated downregulation of Bcl2 in their MBC; in the other 2 Bcl2 expression was retained. Of the 4 Luminal A cases which remained ER positive in their MBC, 2 maintained Bcl2 expression in their MBC, one lost Bcl2 expression in its MBC, while in one Bcl2 was upregulated in a subset of MBC showing increased PR expression. Of the 6 ER- cases, 3 remained Bcl2 negative in all MBC, while 3 demonstrated focal upregulation in a minority of MBC. The Luminal B case retained weak ER labeling and Bcl2 expression in its MBC.

Conclusions: Bcl2 is uncommonly and inconsistently overexpressed in MBC. Instead, **downregulation** of Bcl2 expression may occur in the setting of hormone therapy resistance. Since Bcl2 may paradoxically inhibit cell growth in some solid tumors, we hypothesize that downregulation of Bcl2 may promote MBC growth. Our findings call into question the utility of potential anti-Bcl2 targeted therapy in MBC.

307 Mastectomy Offers the Most Effective Local Surgical Control for Ductal Carcinoma In Situ (DCIS)
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Background: Successful breast-conserving therapy for DCIS is limited by high rates of residual disease and the need for radiotherapy and/or re-excision for better local disease control. This study aimed to identify clinical, surgical, and pathological factors associated with negative surgical margin in re-excised breast specimens.

Design: Forty-seven cases of pure DCIS cases with 2 or more surgical resections were selected from the files of the Department of Pathology. All caes had local resection as the first procedure except for 2 cases with simple mastectomy. All had at least one positive or close (<1mm) margin in the first surgical specimens. 44 cases had 2 procedures, 2 cases had three, and 1 case had four. Clinical and pathological informations including patients' age, grossly identifiable lesion, tumor size, tumor grade, ER and PR status, background proliferative changes (ADH and DH), and time intervals between the first and last surgical procedures were reviewed and analyzed.

Results: Among the 47 cases at the last surgical specimens (17 mastectomies and 30 local re-excisions), only 5 did not show residual DCIS and 19 archived negative margin (>10 mm or no tumor in the specimen). For the rest of the 23 cases, 7 had positive margin (tumor on inked), 8 had close margin (< or = 1 mm), 7 had near margin (>1, but < or =3 mm), and one had a 4 mm margin. Mastectomy was most effective in predicting negative margins (94.12%) compared to local re-excision (30%) in both univariate (p<0.0001) and multivariate (p=0.0053) analysis. The time intervals between the initial and last procedures also seem to be critical for margin control (p=0.0173) in univariate analysis, with a longer interval associated with negative margin. Also, the presence of a grossly identifiable lesion was associated with better margin control (100% vs. 42.62%, p=0.0518). Other factors including age, tumor size, nuclear grades, ER and PR status, and background proliferative changes (ADH and DH) did not affect the margin status.

Conclusions: The majority of the re-excision specimens for DCIS with close margins will have residual disease. Mastectomy, intervals between first and last surgery, and gross mass lesions are most important predictive factors for negative margins.

308 Lobular Carcinoma In Situ Variants Misinterpreted as Ductal Carcinoma In Situ in Core Biopsy: Incidence and Significance
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Background: Differentiating ductal (DCIS) vs. lobular carcinoma in situ (LCIS) on core biopsy (CB) has important clinical implications. Recently described LCIS variants, such as LCIS with necrosis (LCIS-N) and pleomorphic LCIS (LCIS-P), share some morphologic and biologic features with DCIS and can make this morphologic distinction more difficult. However, E-cadherin (EC) has been shown to be helpful in borderline cases. In this study, we performed EC immunostains on CB with a diagnosis (DX) of solid DCIS to determine the incidence of LCIS misdiagnosed as DCIS and reviewed the clinical information to determine the significance.

Design: Consecutive CB with an original DX of predominantly solid DCIS, with or without invasive carcinoma (IC), performed between 1/03 and 12/05 were included (N=139). Upon review cases with significant micropapillary or cribriform architecture or insufficient remaining tissue were dropped. EC was performed on the 82 remaining CB. On review, the DX of LCIS was based on EC negativity as well as morphology. LCIS-P DX required LCIS cells with cellular pleomorphism and nuclear size >3.5x a lymphocyte. In LCIS-N the CIS cells with classic lobular cytology displayed luminal necrosis. Subsequent surgical excision (SSE) was reviewed in selected cases.

Results: In 6 cases the tissue was insufficient for EC interpretation. Remaining cases (N=76) included 55 CIS without and 21 with associated IC. Upon review of HE and EC, 18/76 (24%) of all solid DCIS (with or without IC) was reclassified as LCIS including 9 variants (3 LCIS-P, 6 LCIS-N) and 9 classic LCIS (LCIS-C). In all the reclassified cases (with and without IC) the most common original DX was grade 1 solid DCIS. Overall, 35% (12/34) of grade 1, 18% (4/22) of grade 2 and 10% (2/20) of grade 3 solid DCIS was EC negative.

Cases Reclassified as LCIS

Original Diagnosis (w/o IC)	LCIS-N	LCIS-P	LCIS-C
Grade 1 (N=7)	0	0	1
Grade 2 (N=32)	5	1	0
Grade 3 (N=16)	0	0	0

In the group of DCIS without associated IC, the DX was changed to LCIS variant in 11% (6/55) cases. Two were upstaged to invasive lobular carcinoma in SSE. A single case of LCIS-C had unwarranted SSE (1/55, 2%).

Conclusions: In the three years included in the study, 13% of solid DCIS diagnosed in CB without IC had DX revised to LCIS. The majority of these were LCIS variants requiring complete excision like DCIS, therefore surgical management was unaffected. However, this distinction has treatment implications as radiation therapy is not indicated for any LCIS.

309 Flat Epithelial Atypia on Core Biopsy and Subsequent Surgical Excision: A Five Year Experience
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Background: Flat epithelial atypia (FEA) of the breast is a problematic and often subtle lesion. Though observational data have shown an association between FEA and synchronous atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS), invasive carcinoma (IC) particularly tubular carcinoma, and lobular neoplasia, a lack of standardized nomenclature, clear objective diagnostic criteria, or certainty about the natural history of FEA have contributed to a lack of standardized recommendations for subsequent surgical excision (SSE) when FEA is identified on core biopsy (CB). In this retrospective review, we report our five-year experience with FEA diagnosed on CB and findings at SSE at an academic medical center (Northwestern University Feinberg School of Medicine).

Design: A search of pathology records revealed 8712 breast CB samples received between 01/03 and 08/08. Of these, FEA (or equivalent diagnosis with variant nomenclature) was among the diagnoses rendered in 812 (9.3%) cases. Cases with IC, DCIS or ADH in the same CB sample were excluded, leaving 56 cases for review. Subsequent breast pathology specimens obtained at our institution, patient age and basic imaging information were obtained from pathology records, and original CB and SSE slides were reviewed.

Results: Of 56 CB cases with FEA, SSE information was available in 42 (75%; 4 mastectomies, 38 lumpectomies). Of these 42 cases, 14 (33%) were shown to have concurrent more significant lesions requiring SSE (ADH, DCIS or IC) as diagnosed by additional biopsy(ies) in the same (N=9) or contralateral (N=5) breast; IC was found at SSE in 3/14 cases and DCIS was found in 3/14 cases. 4 of the 42 (9.5%) cases had atypical lobular hyperplasia (ALH) or lobular carcinoma in situ (LCIS) diagnosed in the same biopsy; none of these had DCIS or IC at SSE. Of the remaining 24 cases (57%), with no more significant lesions than FEA on CB, only one case was upgraded to DCIS (grade 1) at SSE, and none were found to have IC.

Conclusions: FEA is challenging to diagnose and manage. As FEA becomes more frequently encountered on CB (due in part to imaging advances), delineation of guidelines for subsequent management is important. In our 5 year experience, 1/28 (3.6%) cases of FEA diagnosed on CB, with no concurrent more significant lesion, was upgraded to DCIS at SSE. This reinforces the need for diligent search (by pathology and imaging) for synchronous more significant lesions and suggests that close clinical follow-up may be a reasonable management strategy for FEA alone diagnosed on CB.

310 Triple Negative Breast Cancers and Basal Immunophenotypic Expression
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Background: Triple negative (TN) breast cancers are aggressive tumors defined by absence of estrogen and progesterone receptor (ER,PR) and c-erbB2 expression. Between 12% to 24% of breast cancers are TN, with many expressing basal markers on immunohistochemistry. In this study, we evaluate their clinicopathologic characteristics and determine immunohistochemical expression of basal markers (CK5/6, CK14, CK17), SMA, p63, CD117 and EGFR.

Design: Archival files of the Department of Pathology, Singapore General Hospital, were searched for cases of TN breast cancers diagnosed between 2003 and 2006. Representative tumor areas were selected for tissue microarray (TMA) construction using 2 mm cores. Antibodies to CK5/6, CK14, CK17, p63, CD117 and EGFR were applied to TMA sections using the streptavidin-biotin method. Cytoplasmic staining for CK5/6, CK14, CK17, SMA, CD117; nuclear reactivity for p63 and cytoplasmic membrane positivity for EGFR were noted. Intensity (0, 1+, 2+, 3+), proportion of tumor cells stained, and intensity-percentage scores (IPS), were documented. Results were correlated with clinicopathologic parameters. A p value <0.05 defined statistical significance.

Results: Median age of 177 women with TN breast cancers was 53 years. Majority (85.3%) were Chinese, 4.5% Malay, 9% Indian, and 1.1% of other ethnic origins. Tumor size ranged from 0.5 to 20 cm (median 2.5 cm). Infiltrative ductal carcinoma was the commonest subtype (92.1%). Histologic grade 3 tumors predominated (80.8%), with grades 2 and 1 forming 11.9% and 1.7% respectively. Ductal carcinoma in situ (DCIS) was present in 45.7% of cases, and 83.9% were high nuclear grade. Node positivity occurred in 35.1%. Basal markers CK5/6, CK14 and CK17 were expressed in 16.4%, 54.2% and 80.6% respectively. SMA, CD117, p63 and EGFR were positive in 31.6%, 66.7%, 43.5% and 35% of cases. SMA intensity correlated with larger tumors and lower incidence of accompanying DCIS, while its IPS was related to the presence of lymphovascular invasion. P63 immunostaining intensity was associated with presence of DCIS. No other significant correlations were found.

Conclusions: TN breast cancers in our Asian population are high grade T2 tumors. Among basal markers, CK5/6 is the least and CK17 the most sensitive in demonstrating a basal phenotype. Our findings indicate that TN tumors cannot be directly equated with a basal phenotype. CD117 and EGFR are potential oncologic targets that may offer additional management options in this group of tumors. The presence of SMA and p63 immunostaining reflects possible myoepithelial differentiation.

311 The Relationship between Oncotype DX Recurrence Score, Clinicopathological Factors, and Molecular Classification in Breast Cancer

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Background: Oncotype DX has been increasingly used to aid adjuvant treatment decisions in breast cancer management. In current study, we sought to investigate the relationship between Oncotype DX recurrence scores (RS), clinicopathological features, and molecular classification in invasive breast carcinomas.

Design: We identified 51 infiltrating carcinomas (48 IDC and 3 ILC) from our departmental file and analyzed the relationship between RS and clinicopathological factors. 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 as follws: Luminal A--ER+, HER2-, EGFR and CK5/6+/-; Luminal B--ER+, HER2+, EGFR and CK5/6 +/-; HER2--ER+, HER2+, EGFR and CK5/6 +/-; Basal-like--ER-, HER2-, EGFR and/or CK5/6 +; unclassified --all 4 markers neggative.

Results: Among the 51 cases, 29 had low RS (0-17), 19 had intermediate RS (18-30), and 3 had high RS (>31). PR expression was inversely associated with RS (mean Allred scores =7.76, 6.47 and 2.00 in low, intermediate and high RS group; p=0.0008). Nuclear grade was also associated with RS: 96.5% of the low and 89.5% of the intermediate RS groups were grades 1 and 2, while 100% of the high RS group was grade 3 (p=0.0238). Of the three HER2 positive cases, one was in the intermediate RS group and two were in the high RS group. EGFR and CK5/6 were negative in all cases. All 29 cases of the low RS group belonged to luminal A subtype; 18 of the intermediate RS group were Luminal A, and 1 was luminal B; 3 high RS cases had 2 in Luminal B subtype and 1 in unclassified subtype. Patients' age, tumor size, presence of DCIS, mitosis, and expression of ER and Ki-67 did not show correlation with RS.

Conclusions: PR negativity is strongly correlated with a higher RS score, as well as HER2 over expression and Luminal B subtype, while the luminal A subgroup is associated with a lower RS score. More studies are needed to further investigate these relationships.

312 Expression of FOXA1, Estrogen Receptor Associated Transcription Factor, Correlates with Oncotype Dx Recurrence Score

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Background: FOXA1 is a forkhead family transcription factor expressed in breast cancer cells. We, in addition to others, have reported that FOXA1 expression is associated with good prognosis in breast cancer and it correlates with luminal A subtype. Oncotype Dx® assay (ODx) is a commonly used prognostic tool used in ER positive/Node negative breast cancer patients. Recurrence Score (RS) obtained in this test can classify patients in low, intermediate and high risk groups. We investigated whether FOXA1 expression and RS identify the same patient population and whether its expression can serve as a cheaper surrogate for ODx.

Design: We selected 130 cases from Indiana University affiliated hospitals in which ODx assay was performed between years 2005 to 2008. Blocks on which ODx assay was performed were available in 79 cases. IHC was performed on the same block using FOXA1 antibody sc-6553 (Santa Cruz Biotechnology). Briefly, 4 µm sections after hydration and antigen retrieval were incubated with goat-anti-human FOXA1 antibody (1:250). The reaction was visualized with anti-goat HRP polymer conjugate (Invitrogen) using DAB plus (Dako) and haematoxylin QS (Vector Laboratories, Burlingame, CA, USA) counterstain. The specificity of staining was verified using non-immune goat serum and PBS controls. Percentage (P) and intensity (I) of nuclear expression were multiplied to generate numerical score (S=P x I). A score of more than 30 is considered positive. Data were analyzed using SPSS 16.0 software.

Results: All patients (n=79) in the study were ER positive and node negative. Patient characteristics are described in table 1. FOXA1 expression correlated negatively with RS (p=0.002) and tumor grade (p=0.004) and with tumor type; higher expression in lobular compared to ductal cancers (p<0.0009). RS also correlated with positivity with grade (p=0.037) and tumor type, higher score in ductal cancers (p=0.047). Correlation between FOXA1 expression and RS remained significant even after adjusting for tumor type (p=0.003) or grade (p=0.031).

Conclusions: FOXA1 expression correlates inversely with RS and identifies the same group of patients. Both these prognostic markers aim to identify low risk ER positive node negative patients who can be spared of toxic chemotherapy; these markers can potentially be used interchangeably in clinic if further validation studies confirm our findings.

313 Identification of Cancer-Specific Glycoproteins in Triple-Negative Breast Cancer

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Background: Breast cancer is a heterogeneous group of tumors and can be subdivided into subtypes on the basis of expression of estrogen receptor (ER) and progesterone receptor (PR) and HER2 gene amplification. The expression patterns of these growth factor receptors (GFRs) are the key prognosis factors for breast cancer therapy such as hormone therapy, biological therapy, or chemotherapy. Breast cancer without ER, PR, and Her2 expression (Triple-negative breast cancer, TNBC) is associated with more aggressive clinical courses, limited treatment options, and worse clinical outcomes. Patients with TNBC are insensitive to anti-estrogen or anti-HER2 therapy. Over

expression of other cell surface proteins and increased signaling of other GFRs have been indicated to contribute to the resistance. Identification and better understanding of cell surface proteins over expressed in TNBC could be used to develop or guide novel therapies and improve clinical outcomes.

Design: Proteins exposed to extracellular environments are mostly glycosylated; therefore we isolate glycoproteins using solid-phase extraction of glycopeptides from TNBC and patient-matched non-cancer tissues. The isolated glycopeptides were analyzed by LTQ and LTQ Orbitrap mass spectrometers and quantified by automated label-free differential expression software, SIEVE. Over expression of cell surface proteins were further verified by selective reaction monitoring (SRM), Western blot analyses, and/or immunohistochemistry.

Results: Five patient-matched TNBC and non-cancer controls were analyzed. 313 glycopeptides from 145 glycoproteins were identified. Candidate glycoproteins with biological significance were found to have increased expression in cancer tissues. In the future, larger number of specimens will be used to validate these findings.

Conclusions: This study identified over a hundred potential glycoproteins over expressed in TNBC using glycoproteomic approach. These proteins may be potential candidate biomarkers for targeted therapy in this special group of breast cancer patients.

314 Feasibility and Potential Utility of IPX Cocktail Double Stain in Breast Cancer

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Background: The usefullness of cocktail antibody (cytokeratin 903, P63 and racemase) with double stain in the diagnosis of prostatic adenocarcinoma inspires the desire to develop a similar antibody cocktail for breast cancer. Although no equivalent known markers differentiating benign versus neoplastic epithelium, lacking of myoepithelial cells is a diagnostic feature of invasive mammary carcinoma. This study evaluated a novel IPX cocktail double stain in the diagnosis of breast cancer.

Design: IPX (Biocare, Concord, CA) composed of primary antibodies cytokeratin 5 (CK5), CK14 and p63 and secondary antibody CK18. Thirteen mastectomy or lumpectomy specimens were retrospectively reviewed to identify invasive or in situ carcinoma, atypical or usual ductal hyperplasia (ADH or UDH), adenosis and normal breast. 4-um sections were obtained from selected formalin fixed and paraffin embedded blocks to stain with IPX cocktail following the manufactures recommendation. Appropriate negative and positive controls, and p63 stain were performed in parallel.

Results: Nineteen tissue sections selected consisting invasive ductal carcinoma (12), invasive lobular carcinoma (1), ductal carcinoma in situ (12), ADH (1), UDH (2), adenosis (2) and normal breast (19). CK18 stained the cytoplasm of the epithelium uniformly red without discrimination of benign versus malignant component. It was useful in identifying small foci of epithelial cells or cells arranged in single file, especially when there was prominent lymphocytic infiltration or desmoplastic background masking the foci of interest. The myoepithelial markers stained the nuclei (P63) and cytoplasm/membrane (CK5 and CK14) brown contrasting nicely against the red epithelial component. It better highlighted the myoepithelial cells than p63 stain alone. The invasive carcinoma completely lacks the brown stain; in situ tumor stained at the peripheral/basal layer. Basal and intraparenchymal stain were seen in DH, adenosis and normal tubules/ductules with substantially less intraparenchymal staining in ADH.

Conclusions: IPX is an adequate stain with minimal background interference and high specificity in breast tissue. It can be used in diagnose of breast carcinomas with the advantage of simultaneously testing of multiple markers on one tissue section, which allows conservation of valuable tissue of limited biopsy for prognostic/predictive marker testing. It is advantageous in identify small foci of tumor and aid in definitive diagnosis of microinvasion.

315 Aberrant WT-1 Expression in Myoepithelial Cells of Inflammatory Breast Cancer

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Background: Our previous studies revealed that Wilms' tumor 1 (WT-1) protein was co-expressed with smooth muscle actin (SMA) and CD31 in over 90% of the ME and endothelial cells in normal and common types of breast lesions (Li and Man. Cancer Biomarker, in press). As WT-1 has been reported to have significant paracrine inhibitory functions on tumor cell growth, our current study attempted to assess the expression status of WT-1 in ME cells of normal and pre-invasive ducts or acini adjacent to inflammatory breast cancer, a rare but one of the most aggressive form of breast malignancies.

Design: Sets of consecutive sections from 20 inflammatory human breast cancer and 20-common forms of breast malignancies were subjected to double immunohistochemistry with monoclonal antibodies to WT-1 (6F-H2; Cell Marque, Rocklin, CA), SMA, CD31, and CD34. From each case, 4-5 randomly selected duct or acinar clusters were photographed, and enlarged prints were made. The numbers of positive and negative ME and endothelial cells for each molecule were counted, scored, and statistically compared with the Pearson's Chi-squared test.

Results: In common forms of breast lesions, over 90% of the ME and endothelial cells co-expressed WT-1 and SMA or CD31. In inflammatory breast cancer, over 80% of the ME cells with strong SMA immunostaining were devoid of WT-1 expression in 19 of the 20 cases. In addition, most morphologically distinct micro-vessels that were strongly immunoreactive to CD31 or CD34 were either devoid of, or had substantially reduced, WT-1 immunoreactivities. In one case of inflammatory breast cancers, tumor cells within the lymphatic channels were strongly positive for WT-1.

Conclusions: These findings suggest that WT-1 may be an important tumor suppressor, and the loss of WT-1 in ME cell layers may lead to the aggressive behavior in epithelial cells. These findings also suggest that patients with inflammatory breast cancer may

carry WT-1 related genetic alterations (Supported in part by grants DAMD17-01-1-0129, DAMD17-01-1-0130, PC051308 from Congressionally Directed Medical Research Programs, BCTR0706983 from The Susan G. Komen Breast Cancer Foundation to Dr. Yan-gao Man, and 2006CB910505 from the Ministry of Chinese Science and Technology Department to Drs. Xichen Zhang, Yan-gao Man, and Guiyuan Li).

316 Clinical Analysis of Mucocele-Like Tumors of the Breast: Analysis of a Large Benign Breast Disease Cohort

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Background: Mucocele-like tumors (MLT) of the breast are unusual lesions characterized by cysts distended with mucin that also dissects/extravasates through the epithelium into surrounding stroma. They are accompanied by variable epithelial proliferation, with an increased frequency of associated atypical ductal hyperplasia (ADH). It is not known whether MLT represent a risk factor for subsequent development of breast carcinoma.

Design: Our benign breast disease cohort is comprised of 9376 women who underwent excisional breast biopsy from 1967-1991. Slides from all patients were reviewed retrospectively in a blinded fashion and classified per standard diagnostic criteria by two study pathologists. Mean follow up is 13.7years. We analyzed subjects with MLT diagnoses for the frequency of proliferative lesions, including ALH/ADH, and for their likelihood of developing breast cancer.

Results: The cohort contained 70 MLT (0.75%). Thirty patients (42.9%) were >55yrs of age at time of diagnosis, 24 (34.3%) were 45-54yrs and 16 (22.9%) were <45yrs. MLT were more often associated with proliferative lesions (70% in MLT vs 33% for the cohort overall). ALH/ADH was present in 21.4% MLT lesions, vs 3.4% in the cohort overall (p<0.0001). To date, 6/70 patients with MLT (8.6%) have developed breast carcinoma; this frequency is not significantly different than the BBD cohort overall (p=0.8780).

Conclusions: Our findings support previous studies showing a relationship between MLT and atypia. However, beyond the risk associated with atypia itself, we do not observe an additional risk of breast carcinoma associated with the presence of the MLT.

317 Diagnosing Breast Cancer Using Multimodal Spectroscopic

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Background: Despite recent advances,there remain challenges to early diagnosis of breast cancer.Among these are mammographically suspect lesions that are benign on biopsy and positive margins at breast cancer surgery that necessitate reoperation for complete resection. Our goal is to develop optical spectroscopy techniques to 1)diagnose benign and malignant breast lesions *in vivo* in real-time without tissue excision and 2)confirm status of margins of resection during breast cancer surgery.

Design: We conducted an *ex vivo* clinical study to determine the feasibility of diagnosing breast cancer using multimodal spectroscopy (MMS) - a combination of Raman,diffuse reflectance (DRS), and intrinsic fluorescence spectroscopy (IFS). These spectroscopic techniques provide complementary information.Raman spectra provide specific information about the chemical composition of tissue and are fit using a linear combination of Raman active tissue components (calcium,collagen,lipids,etc.).DRS spectra are modeled using diffusion theory and provide information about tissue absorbers and scatterers.IFS spectra are extracted from the combined fluorescence and DRS spectra, and provide information about tissue fluorophores (collagen,NADH,etc.).In the *ex vivo* study,we obtained 378 sets of spectra from fresh breast excisional biopsy specimens from 48 patients,using a novel portable MMS instrument designed for use in clinical settings that is capable of acquiring reflectance,fluorescence and Raman spectra via a single optical fiber probe.Data were collected from specimens within 30 minutes of surgical excision.

Results: After spectroscopic acquisition,breast specimens were submitted for routine pathology evaluation,performed by an experienced breast pathologist blinded to the spectroscopy results.Spectroscopy results were compared to pathology diagnosis, and an MMS diagnostic algorithm developed using spectral fit parameters.A previously devised Raman diagnostic algorithm was also applied prospectively to this data set with sensitivity,specificity,PPV and NPV and test efficiency of 78%,98%,82%,98%,and 86%,respectively,for the diagnosis of invasive breast cancer.

Conclusions: The combination of Raman,DRS,and IFS yields promising results for discrimination of breast cancer from benign breast lesions via objective,quantitative,physically meaningful parameters.

318 Comparison of Gene Expression Profiles of Areas of Micropapillary (MP) Differentiation of Invasive Breast Carcinomas with Focal MP features with Usual Ductal (No Special Type, NST) Components

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Background: Invasive breast carcinomas with MP features are aggressive tumors frequently associated with lymphatic invasion and nodal metastasis. Although areas of MP differentiation may be very focal within breast cancers, metastatic tumor foci often show similar morphologic features in these cases, suggesting that they arise from these limited areas. We hypothesized that comparison of the gene expression profiles of MP areas to NST components of the same tumors may help identify genes that play a significant role in lymphatic tumor spread.

Design: MP and NST areas of 16 archived formalin-fixed paraffin-embedded (FFPE) invasive breast carcinomas with focal MP features were macrodissected separately 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. The 12 highest quality sample pairs 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 from FFPE tissues. In addition, 13 genes with known involvement in breast cancer pathogenesis were also quantified by TaqMan q-RT-PCR in the same samples.

Results: Areas of MP features ranged between 5 to 50% in the tumors. Comparison of whole genome gene expression profiles of MP versus NST areas of the tumors revealed 25 differentially expressed genes at a significance level of p<0.001. Among the 13 genes evaluated separately by TaqMan q-RT-PCR, we identified Tenascin C, a matrix protein involved in metastasis, to be expressed differentially between MP and NST areas of the tumors. Verification of whole genome DASL and TaqMan results are being carried out by immunohistochemistry.

Conclusions: Our results suggest that focal areas of MP differentiation in invasive breast carcinomas show differences in their gene expression profiles compared to NST areas of the same tumors. Identification of differentially expressed genes in MP areas may help identify genes and/or proteins that play a significant role in the metastatic spread of these aggressive tumors.

319 Histopathological Analysis of Nipple Involvement by Breast Carcinomas in Mastectomy Specimens from a Single Institution

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Background: Mastectomy has become less popular among breast cancer patients and surgeons due to effective local control from breast conserving surgery. One of the most important arguments of using mastectomy is to eliminate possible residual tumor involving nipple. Here we analyzed the rates and types of nipple involvement and identified factors that are most strongly associated with disease affecting nipple.

Design: Five hundred thirty-nine mastectomies from the file of the Department of Pathology between 1997 and 2007 were identified. Clinical and pathological factors including patients' age, tumor location, tumor type, uni- or multifocality, tumor size, histological grade, nuclear grade, expression of ER, PR and HER2, margin status, and lymph node status were reviewed and recorded.

Results: Among the 539 breast carcinomas with mastectomies (114 of DCIS, 356 of IDC, 43 of ILC, 22 of IDC with ILC, and 4 of LCIS), 83 (15.4%) cases demonstrated nipple involvement. Nipple involvement was most significantly associated with tumor location (central 33% and 4 quadrants 45% vs 1-3 quadrants 7%-13%; p<0.0001), tumor size (29% in tumors > 5cm vs. 11-15% in tumors < 5cm; p=0.0004), and lymph node status (21% in node positive tumors vs. 11% in node negative tumors; p=0.007). The types of nipple involvement includes DCIS (25 cases), IDC (28 cases), Paget's disease (20 cases), lobular carcinomas (9 cases) and dermal lymphatic invasion (1 case, not included in the calculation). The types of nipple involvement were significantly associated with tumor types (p<0.0001), nuclear grades (p=0.0007), along with tumor location (p<0.0001), tumor size (p<0.0001), and lymph node status (p<0.0001). The nipple involvement and types of involvement were not associated with patients' age, uni-or multifocality, histological grade, status of ER, PR and HER2, and status of margin.

Conclusions: Nipple involvement by breast carcinomas is strongly associated with tumor location, tumor size, and lymph node status. Patients with high risk for nipple involvement should be treated accordingly.

320 Expression of Insulin-Like Growth Factor-I Receptor (IGF-IR) in Normal Breast Tissue and Breast Cancer Risk: Results from the Nurses' Health Study

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Background: IGF-IR is a transmembrane tyrosine kinase receptor activated by binding with insulin-like growth factor-I (IGF-I). Prior studies support a role for the IGF-I/IGF-IR pathway in breast cancer development and progression, and elevated serum IGF-I has been associated with an increased breast cancer risk. However, a possible association between IGF-IR expression in normal breast tissue and risk of subsequent breast cancer has not been previously evaluated.

Design: We conducted a case-control study of benign breast disease (BBD) and breast cancer risk nested within the Nurses' Health Study. Tissue microarrays (TMA) containing normal terminal duct lobular units (TDLUs) were constructed from 240 benign breast biopsies with available tissue blocks (59 cases; 181 controls). TMA sections were immunostained for IGF-IR and assessed blinded to case/control status for membrane and cytoplasmic expression in normal TDLUs. Odds ratios (OR) and 95% confidence intervals (CI) were calculated for the association between IGF-IR expression and subsequent breast cancer risk adjusting for age and BBD category.

Results: There was no relationship between membranous expression of IGF-IR in normal TDLU epithelial cells and subsequent breast cancer risk (OR 0.84; 95% CI 0.51-1.38). In contrast, cytoplasmic IGF-IR expression was associated with an almost 2-fold increase in risk for subsequent breast cancer (OR 1.89; 95% CI 1.19-3.01). Among the small subset of women in whom the normal epithelium showed cytoplasmic staining for IGF-IR in the absence of membrane staining, the OR for subsequent breast cancer was 4.26, but the 95% confidence interval was broad (1.41-12.81).

Conclusions: Among women with biopsy-proven BBD, cytoplasmic expression of IGF-IR in normal breast epithelial cells was associated with an almost 2-fold increase in breast cancer risk, independent of BBD category. This finding raises the possibility that blocking IGF-I/IGF-IR signaling may represent a new breast cancer prevention strategy.

321   Estrogen/Progesterone Receptor (ER/PR) and HER2 Expression Profiles in Breast Cancers (BCs) with Isolated Bone Metastasis (IBM) Are Different from Those in BCs with Metastasis to Other Sites
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Background: Bone is a common site of relapse after treatment for primary BC and up to 90% of patients with terminal BC have bone metastasis. The aim of this study was to identify the clinical and pathological features of BC with IBM and compare them to those in cases with metastasis at other sites.
Design: The tumor registry at the authors' institution was searched to identify cases of BC with associated metastasis. These were then classified into cases with IBM, isolated or multiple visceral organ (e.g. lung, liver, ovary) metastasis (IVM or MVM), central nervous system metastasis (CNSM) [isolated (ICNSM) or combined with visceral organ(s)], or with multiple metastases (MM) of any combination. The status of ER/PR and HER2 expression and the time to relapse were then compared between the different groups.
Results: Of 2,738 patients with BC diagnosed between 1997 and 2003, 356 patients had metastatic disease at diagnosis or later. The proportion of cases with IBM that expressed ER/PR (52/59; 88%) was higher than that in cases with MM (50/96; 52%) (p<0.0001) and than that in cases with non-bone metastases (49/81; 60%) (p<0.001), including cases with IVM (28/49; 57%) (p<0.001), MVM (9/15; 60%) (p<0.0001), and ICNSM (12/17; 71%) (p=0.1). The distribution of BC molecular subtypes in cases of IBM was significantly different from that in the remainder.

Metastatic organs	Luminal A type (ER/PR+/HER2-)	Luminal B type (ER/PR+/HER2+)	HER2 type (ER/PR-/HER2+)	Triple negative (ER/PR-/HER2-)	P value (vs. IBM)
IBM	18	18	0	2	
IVM	16	7	9	9	0.0002
MVM	5	1	2	5	0.0003
CNSM	3	5	2	3	0.02

Of 203 originally metastasis-free patients, 38, 30, 12 and 80 eventually developed IBM, IVM, ICNSM, or MM, respectively. The mean time to relapse in cases with IBM (40.2 mo) was slightly less than that in cases with IVM (44.2 mo) (p>0.05) but more than that in cases with ICNSM (18.8 mo) (p<0.05) and MM (31.0 mo) (p<0.05).
Conclusions: The ER/PR and HER2 expression profiles of BC patients with IBM are different from than those in patients with non-bone metastasis or those with MM. Although it is unclear at this point how the expression of these markers is affecting the distribution of distant metastasis, such differences could potentially be utilized in determining the most appropriate survey mechanism for follow up of patients with non-metastatic disease. Evaluation of additional clinical and pathological differences between these groups is ongoing.

322   Metaplastic Breast Carcinomas Are Basal-Like Breast Cancers: A Genomic Profiling Analysis
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Background: Metaplastic breast carcinomas (MBCs) comprise a group of aggressive and chemotherapy resistant cancers characterised by neoplastic cells displaying differentiation towards squamous epithelium or mesenchymal elements. Previous histopathological and immunohistochemical analysis of MBCs suggested that these cancers would have a basal-like profile.
Design: We investigated the molecular subtype of 20 MBCs using microarray-based expression profiling data. These expression data were compared to those of 79 invasive ductal carcinomas (IDCs) of basal-like phenotype by unsupervised hierarchical clustering, supervised analysis and gene pathway analysis.
Results: We demonstrate that 95% of all MBCs are of basal-like molecular subtype. Furthermore, unsupervised hierarchical clustering analysis and pathway analysis of the profiles of MBCs revealed that MBCs are part of the spectrum of basal-like breast cancers. Significance analysis of microarrays (SAM) identified 1385 transcripts differentially expressed between MBCs and IDCs of basal-like phenotype. Pathway analysis using these genes revealed that DNA repair pathways, including BRCA1 pathway, PTEN, a gene whose loss of function is associated with resistance to chemotherapy, and TOP2A, the molecular target of anthracyclines, are significantly downregulated in MBCs compared to basal-like IDCs. These findings may at least in part explain the reported poor responses to chemotherapy of MBCs. Furthermore, MBCs showed significantly higher expression of genes related to myoepithelial differentiation and epithelial to mesenchymal transition.
Conclusions: Our results demonstrate that MBCs are part of the spectrum of basal-like breast carcinomas and display a myoepithelial and EMT-like molecular make-up. The reported poorer response to chemotherapeutic agents in patients with MBCs may stem from downregulated DNA damage response pathways, PTEN and TOP2A.

323   Estrogen Receptor (ER) and Androgen Receptor (AR) in Normal Human Breast Tissue and Breast Carcinoma
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Background: While AR, the same as ER, is strongly expressed in majority of breast carcinomas, little is currently known about the role of AR in breast carcinogenesis. The study of AR and its association with ER in normal breast tissue and comparison with the AR/ER status in invasive carcinoma might be revealing.
Design: Twenty breast core biopsies were identified: 10 from patients age 49 or younger, 10 from 50 or older; (10 benign biopsies, and 10 with breast cancer). Immunohistochemical (IHC) stain for AR and ER was performed on consecutive sections, and double stain for AR/ER was also performed on the same biopsies. 97 invasive breast carcinoma cases with complete ER/AR information were identified.
Results: In general, there were scattered AR positive cells in benign breast tissue, with

the same distribution pattern as ER, as shown by individual IHC stain on consecutive sections. The percentage of AR and/or ER positive cells in the terminal ductal lobular units (TDLU) varied considerably from scattered (less than 10%, mostly in lobules), to modest (around 50%, mainly in terminal ducts), to diffusely positive (more than 90%, mainly in ducts showing somewhat columna/round cell changes). This was true regardless of the age groups or the presence or absence of breast carcinoma. Interestly, double stain shows that almost all ER positive benign luminal cells are also AR positve. While there are often AR positive only luminal cells (around 5-10%), ER positive only cells were rare. In invasive carcinoma, 55 cases are ER+/AR+ (49 low grade, 6 high grade), 21 ER-/AR- (20 high grade, 1 low grade), 17 ER-/AR+ (16 high grade, 1 low grade), only 5 ER+/AR- (4 low grade, 1 high grade). This distribution pattern is in correspondence with the findings published by other researchers.
Conclusions: AR is commonly co-expressed with ER in normal luminal breast epithelial cells. While we speculate that ER positive cells are the progenitors of hormonal carcinogenesis, it is expected to see both ER and AR positive carcinomas, especially in low grade carcinomas. - AR positive only normal luminal cells may serve as the progenitor cells for those ER-/AR+ carcinomas. These tumors are usually high grade, suggesting that other genetic alterations, other than hormonal carcinogenesis, might be involved in its initiation and progression. - ER-/AR+ carcinomas should also be classified as luminal type. It is reasonable to believe that receptor negative carcinomas can also arise from luminal cells, speculating from the presence of large percentage of ER/AR negative luminal cells in TDLU.

324   Proliferative Activity and HER-2 FISH Results on Hormone Receptor/HER2 Positive (Luminal Type-B) Breast Cancer
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Background: An unusual subset of breast carcinoma is characterized by dual activation of hormone receptor (ER and/or PR) and growth factor receptor (HER2) pathways. By gene expression analysis these have been termed "luminal type-B" tumors. As a group they appear intermediate between "luminal type-A" (ER and/or PR positive and HER2 negative) and "HER-2 type" (ER/PR negative and HER2 positive) breast cancer. Our goal was to explore the proliferative rates of luminal type-B carcinomas (in comparison to luminal type-A and HER2 type tumors) and to confirm the HER2 positive status with FISH analysis.
Design: In a companion study we identified fifteen ER/PR/HER2 "triple positive" breast cancers, representing all such cases diagnosed at Hartford Hospital from 2002-2007. To these we added 6 further cases that were ER(+), PR(-) and HER2(+) drawn from the same period. Slides and pathology reports were available and reviewed for all cases including confirmation of the immunohistochemical (IHC) ancillary studies. All 21 cases were referred for MIB-1 IHC and HER-2 FISH analyses. Proliferative indices were then compared to reference file cases of "luminal type-A" and HER2(+) tumors.
Results: The 21 study cases demonstrated proliferative indices ranging from 10-60% (mean = 35.5). This was intermediate between those of 26 reference cases each of "luminal type-A" and HER(+) breast cancers, i.e. 2-25% (mean = 15.6) and 19-90% (mean = 56.5), respectively. FISH analysis confirmed HER2 amplification in 21/21 (100%) of cases (HER2/CEP17 ratio 2.3 – 30.5; mean = 18.1).
Conclusions: Hormone receptor/HER2 positive (luminal type-B) carcinomas occupy an intermediate position between luminal type-A and HER2(+) tumors, not only by IHC receptor analyses but also with regard to proliferative indices. HER2 positivity of such tumors is real including classic "copper pennies" by IHC and amplification by FISH. While it cannot be concluded that this represents a transitional phase between an ER(+)/HER2(-) and ER(-)/HER2(+) phenotype, it remains possible that secondary growth factor receptor activation could, in some cases, be responsible for development of hormone unresponsiveness via crosstalk between downstream moieties of respective hormone and growth factor receptor cascades.

325   Loss of Caveolin –1 Expression in Breast Cancer Associated Fibroblasts Correlates with Tumor Aggressiveness
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Background: Caveolins are the principal protein components of caveolae, which are located at the cell surface . One of the caveolins, caveolin-1 (Cav-1), plays a major role in carcinogenesis through its many functions, such as gene regulation, and signal transduction. Cav-1 was reported to be downregulated during oncogenic transformation of fibroblasts. Using cell cultures established from excised breast cancers, we recently demonstrated that Cav-1 is downregulated in cancer-associated fibroblasts (CAFs), when compared to normal fibroblasts isolated from the same patient. To date, there are no studies addressing the clinical significance of stromal Cav-1 expression in invasive carcinoma of the breast. The aim of this study was to evaluate stromal Cav-1 expression in a large series of invasive breast carcinomas and to examine the association between stromal Cav-1 and clinicopathological variables.
Design: Cav-1 expression was determined by immunohistochemistry using an anti-Cav-1 antibody (Santa Cruz Biotechnology; dilution 1:100) on 160 invasive breast carcinomas, using a tissue microarray. The staining was scored semi-quantitatively as negative (0; no staining), weak (1; either diffuse weak staining or strong staining in less that 30% of stromal cells per core) and strong (2; defined as strong staining of 30% or more of the stromal cells). The median score was used for statistical analysis.
Results: Of the 160 invasive carcinomas, 147 had available cores for evaluation. Strong expression of Cav-1 in CAFs was present in 26 cases, weak in 52, and 47 cases showed an absence of Cav-1 immunostaining in CAFs (some samples could not be scored due to lack of stromal cells). There was a significant correlation of stromal Cav-1 expression

with tumor grade (p=0.017) and lymph node positivity (p=0.05), with a lack of Cav-1 associated with more advanced disease. There was no association between Cav-1 and estrogen, progesterone receptors and HER2.
Conclusions: Loss of Cav-1 expression is CAFs might be a novel marker of tumor aggressiveness in breast cancers.

326 Clinicopathologic Evaluation of Papillary Carcinoma of the Breast
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Background: Intracystic Papillary Carcinoma (IPC) of the breast is traditionally regarded as a variant of ductal carcinoma in situ (DCIS). Recently, Collins and colleagues (*Am J Surg Pathol* 2006) reported lack of myoepithelium (MEC) in 22 IPCs and suggested that this tumor represents an encapsulated invasive carcinoma. Although most studies show that IPC is an indolent neoplasm, reports of lymph node (LN) and distant metastases exist. We evaluated morphology, immunoprofile and clinical follow-up of IPC to address some of these questions.
Design: We searched our files for slides and blocks of IPC diagnosed between January 1990 and June 2008. One pathologist (C.W.) reviewed all available material and assessed tumor morphology, including presence and extent of associated invasive carcinoma (IC). We performed immunoperoxidase stains for MEC (calponin, p63 and smooth muscle myosin heavy chain) on all available tumor blocks, and tested ER, PR and Her2 on one. Clinical data was obtained from medical records.
Results: We identified 23 IPCs from 22 patients (21 women, 1 man), including one with two distinct IPCs. Median age at diagnosis was 70 years (range 47-83), and median tumor size 1.7 cm (range 0.6-5.0). Thirteen tumors involved the right breast, ten the left. The original diagnoses ranged from IC (3), in situ (8), and in situ and IC (12). Seven cases were pure IPC, 4 IPC+microinvasion (MI), 12 IPC+IC≥2 mm. All IC≥2 mm were ductal, well (n=9) or moderately differentiated (n=3); IC was cribriform in one case and mucinous in another. IPCs were predominantly papillary in 17 cases, cribriform in 2 and solid in 4. Associated DCIS was less frequent in pure IPC and IPC+MI (4/11; 36%) than in IPC+IC≥ 2mm (10/12; 83%). All tumors were ER(+), Her2(-); 20/23 were PR(+). Nuclear positivity for p63 highlighted focal residual MEC at the periphery of IPC in 2/23 cases. Three patients underwent mastectomy and 19 lumpectomy. None of 17 patients with LN evaluation (13 sentinel LN and 4 axillary dissection) had metastasis. Two pure IPCs recurred with the same morphology 2 and 10 years after the initial diagnosis.
Conclusions: Our results confirm that most IPCs lack MEC, suggesting an invasive process. Nonetheless, focal residual MEC were present in two cases, raising the possibility that IPC may represent a spectrum of progression from in situ to IC. Our follow-up data confirms that LN involvement is uncommon, and that these tumors have a good prognosis, but can locally recur.

327 Evaluation of HER2 Immunostaining in Breast Carcinoma: Use of Image Analysis in Cases with Indeterminate Scores
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Background: Immunohistochemical staining (IHC) for HER2 in invasive breast cancer (BC) can produce a heterogeneous pattern. It is difficult to assign a definitive score in such cases, especially with values near the cutoff point. For this reason, we include two indeterminate categories ("1+/2+" and "2+/3+") when reporting HER2 results by IHC. At our institution, FISH is routinely performed on all indeterminate cases. This study was aimed to investigate if image analysis could improve scoring accuracy and assist in selection of cases for FISH.
Design: 116 tumors with indeterminate HER2 IHC scores (HercepTest™) and known gene amplification status were re-evaluated using the Aperio Technologies, Inc. ScanScope^XT digital slide scanning system and Spectrum™ software membrane image analysis (IA) algorithm. The original IHC slides were digitally scanned at 20X, and the pathologist outlined all foci of invasive tumor for analysis. IA was performed with the IHC membrane algorithm which scores individual tumor cells as 0, 1, 2 or 3+ based on membrane staining intensity and completeness. 3+ BC had >30% of cells with a score of 3+. 2+ BC had >10% of cells scored 2+. 1+ BC had >10% of cells scored 1+.
Results: By IA, the initial "1+/2+" group was almost equally split into the 1+ (HER2 negative) and 2+ (HER2 equivocal) categories. Half of the cases re-classified as 1+ (18/32; 56%) had staining characteristics near the cutoff point (5-9% cells with 2+ intensity). Importantly, IA correctly identified the HER2-negative subgroup, as none of 32 tumors had evidence of HER2 gene amplification by FISH. Among the initial "2+/3+" cases, only a small proportion (5/39; 13%) was unequivocally positive (3+) by IA.

Corrected IHC scores by image analysis (IA)						
Initial IHC score	1+		2+		3+	
	FISH +	# cases	FISH +	# cases	FISH +	# cases	FISH +
1+/2+ (n=77)	8/77 (10%)	32/77 (42%)	0/32 (0%)	44/77 (57%)	7/44 (16%)	1/77 (1%)*	1/1 (100%)*
2+/3+ (n=39)	31/39 (79%)	2/39 (5%)**	1/2 (50%)	32/39 (82%)	25/32 (78%)	5/39 (13%)	5/5 (100%)

*1 case re-classified as 3+ by IA was confirmed as originally underscored. **2 cases re-classified as 1+ by IA were confirmed as originally overscored.
Conclusions: IA provides a more objective HER2 result and can assist in selection of cases for FISH. Laboratories performing manual analysis should consider assessment of gene amplification whenever there is doubt regarding the appropriate IHC score. This approach should prevent under- and over-assessment of HER2 status.

328 Comparison of Breast Cancers in Hispanic and Non-Hispanic Caucasian Women Using Array Comparative Genomic Hybridization
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Background: In large population studies, Hispanic women are more likely to be diagnosed with higher stage breast cancer and have decreased survival as compared with non-Hispanic Caucasian women. Hypothesized reasons for these discrepancies include biological, socio-economic, environmental and lifestyle variations. Biological differences include hormone receptor status, histological types, and genetic changes. We undertook this analysis to compare breast tumor characteristics at the genomic level in Hispanic and non-Hispanic Caucasian women.
Design: We performed array comparative genomic hybridization on 82 fresh frozen breast carcinomas using an array consisting of over 1100 non-overlapping bacterial artificial chromosome clones. These samples included 54 cases obtained from Hispanic women and 28 cases obtained from non-Hispanic Caucasian women. Genomic gains and losses were determined for each chromosome and the two groups were compared using Fisher's exact test (two tailed).
Results: Common copy number changes in the breast cancers included gains of 1q, 8q, 11q, 12p, 16p, 17q, and 20q, and losses of 5q, 8p, 13q, 16q, and 17p. Comparison of the Hispanic to the non-Hispanic Caucasian cancers showed that only gain of 17q was statistically significant (P= 0.0426).
Conclusions: 17q gain was significantly more common in breast tumors from Hispanic women as compared with non-Hispanic Caucasian women. This chromosomal region includes the *HER2* gene, which was more frequently amplified in the Hispanic group. This finding is may be part of the biological etiology of the previously reported poorer prognosis of Hispanic patients with breast cancer.

329 Multiple Pathological Factors Contribute to Diagnostic Uncertainty in Mammary Low-Grade Adenosquamous Carcinoma. A Review of 35 Consultation Cases
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Background: Low-Grade Adenosquamous Carcinoma (LGASC) is a rare but distinct form of mammary metaplastic tumor. In a retrospective review of LGASC cases seen in consultation, we observed that LGASC nearly always elicited diagnostic uncertainty among referring pathologists even on excisional biopsies. We sought to determine the basis for such uncertainty.
Design: All available histopathological material and archived records from LGASC cases (diagnosed on excisional biopsies, 1999-to date) in our consultation practice were reviewed.
Results: Thirty-five LGASCs were identified among 19,500 consultation cases (0.18%). 30/35 (86%) cases were submitted with an equivocal diagnosis (LGASC was mentioned in differential diagnosis in 3 cases) and 5 cases were submitted as 'positive' (all non-LGASC). All patients were female with unilateral (right: 22, left: 13) and unifocal disease. Mean age of patients was 62.4 (range 34-87) years and mean tumor size was 2.1 (range 0.6-5.0) cm. Based on correspondence, draft report or other communication, diagnostic uncertainty could be attributable to one or more of the following factors: (i) p63 positivity in tumor cell nuclei leading to erroneous interpretation of myoepithelial cells being present (i.e. non-invasive?); (ii) association with a dominant benign lesion including sclerosing papilloma in 14 (40%) cases, radial sclerosing lesion in 5 (14%), and adenomyoepithelioma in 4 (11%); (iii) association with a high-grade spindle cell carcinoma in 6 (17%); (iv) location in nipple or subareolar tissue in 5 (14%), with secondary Paget's disease in 1 case; (v) triple-negativity: ER (-), PR (-) and Her-2/*neu* (-) in an otherwise low-grade tumor; (vi) association with *in situ* carcinoma in 5 (14%), including 3 LCIS. Seven (20%) cases recurred (mean time to recurrence: 40 months, range: 3-108) at the original site with histologically similar tumor (one adenomyoepithelioma with myoepithelial overgrowth recurred as LGASC in 22 mo). Lymph nodes were negative in all 15 cases for which lymph node information was available.
Conclusions: Multiple pathological factors contribute to diagnostic uncertainty in LGASC. These factors include misinterpretation of p63 immunostain, association with a dominant benign lesion or an *in situ* or a higher-grade carcinoma, subareolar or nipple location, and paradoxical triple-negativity in a low-grade tumor. Greater awareness of LGASC and its wide pathological spectrum could reduce diagnostic uncertainty in this tumor.

330 Spectrum of Pathologic Findings in Magnetic Resonance Imaging (MRI)-Guided Breast Core Biopsies
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Background: Breast MRI has been increasingly used in screening high-risk women, and evaluating the ipsilateral and contralateral breasts of women with a newly diagnosed breast cancer. Breast MRI is believed to benefit patients by detecting cancers that may be missed by mammography. However, the increased MRI sensitivity may be associated with a low specficity. This study evaluates the pathologic outcome of MRI-guided breast core biopsies in our institution.
Design: 152 MRI-guided breast biopsies were performed by two experienced radiologists over a 27 month period (5/06-8/08). Abnormal MRI findings included avid, linear, clumped or asymmetric enhancement, and enhancing distortion. The biopsy results were analyzed against the patients' personal and family history of breast cancer, and the biopsy side (ipsilateral vs. contralateral to a diagnosed cancer). Fisher's exact test was used with results considered statistically significant if the p-value was <0.05.

Results: Carcinoma (invasive or in situ) was detected in 14.47% (22/152) patients.

History	H/O breast cancer	H/O ADH	Family H/O breast cancer	No H/O of breast cancer	Total
No. of cases	86	4	30	32	152
Positive MRI bx (%)	15 (17.44%)	0 (0%)	1 (3.45%)	6 (18.75%)	22 (14.47%)

The above table summarizes the rate of malignancy in different groups based on history. The malignancy rate was similar between the group with a history of breast carcinoma and the group without a personal or family history of breast cancer (17.44% vs. 18.75%). Women with a diagnosed breast carcinoma had a higher malignancy rate in the ipsilateral vs. the contralateral breast (11/31 versus 4/38, p=0.013). - Atypical changes were detected in 12/152 cases (7.89%): 9 atypical ductal /lobular hyperplasia (ADH/ALH), 2 ADH bordering on ductal carcinoma in situ (DCIS), and 1 atypical apocrine adenosis. F/U was available in 3 cases. Two ADH bordering on DCIS correlated with DCIS and usual ductal hyperplasia, respectively. One ADH revealed DCIS in subsequent excision. - Benign proliferative changes were detected in 14 cases (9.21%): florid ductal hyperplasia (4), fibroadenoma (7) and papilloma (3). - Nonproliferative fibrocystic changes were detected in 89 cases (58.55%) - Unremarkable breast parenchyma was diagnosed in 15 cases (9.87%).

Conclusions: MRI enhanced breast lesions show a wide spectrum of pathologic findings ranging from invasive carcinoma to unremarkable breast parenchyma. Carcinoma and atypical changes were detected in 22 and 12/152 biopsies, respectively. The remaining 118 cases (77.6 %) were benign.

331 Routine Histologic Parameters Can Predict the Presence of Breast Carcinoma Following a Core Biopsy Showing Atypical Ductal Hyperplasia: A Clinicopathologic Study of 124 Cases

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Background: In this study, we arbitrarily divided atypical ductal hyperplasia of the breast (ADH) into two subtypes, types I and II, diagnosed in the following manner: i) in type I ADH, duct is completely replaced by cells of low-grade carcinoma in-situ but the lesion is smaller than 2 mm, and ii) in type II ADH, ducts were partially involved by DCIS-like cells regardless of the lesional size. The diagnosis of ADH in a core biopsy (CoBx) often leads to an excisional biopsy (ExBx) to exclude carcinoma. Our aim was to derive practical histopathologic parameters, using routine H&E stains, to determine whether histologic subtyping of ADH can predict the presence of carcinoma in the breast.

Design: We retrieved from our institutional pathology archives all cases of ADH diagnosed by CoBx and followed by ExBx during the period from January 1, 2001 to December 31, 2005. CoBx cases were reviewed to confirm the diagnosis and to categorize the lesions into either type I or type II ADH as defined above. The presence of carcinoma, either carcinoma in-situ (DCIS) or invasive carcinoma, in the ExBx specimens was correlated with each type of ADH noted in the prior CoBx. Fisher Exact Test was used to compare the frequencies of carcinoma in the ExBx specimens that were associated with either type I or II ADH.

Results: Of a total of 131 cases, the original CoBx diagnosis of ADH was confirmed in 124 cases. 41 of 124 cases (33.1%) showed either DCIS, invasive ducatal carcinoma (IDC), or both DCIS and IDC in the subsequent ExBx specimens (these were referred to positive ExBx specimens). 33 of 124 CoBx cases (26.6%) fell into our type I ADH, and 91 of 124 CoBx cases (73.4%) into our type II ADH. Positive ExBx specimens followed type I ADH in 26 of 33 cases (78.8%) and followed type II ADH in 15 of 91 cases (16.5%) (p< 0.001). Within the 26 positive cases in type I ADH, 21 showed DCIS alone, 2 IDC alone, and 3 DCIS and IDC. Among the 15 positive cases of type II ADH, 13 exhibited DCIS alone, and 2 DCIS and IDC.

Conclusions: Our so-called type I ADH seen in CoBx is more frequently associated with carcinoma (either in-situ or invasive) in the ExBx specimens than type II ADH. Specifying the histologic subtype of ADH in the pathology report of a core biopsy may prove useful in predicting the presence of carcinoma in the breast.

332 Evaluation of Connexin 43 and 26 in Intraductal Carcinoma and Their Potential Role in Early Mammary Oncogenesis

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Background: Gap junctions (GJ) are tightly packed intercellular channels which allow direct exchange of small molecules between cells. The channels are assembled selectively from a family of >20 proteins called connexin (Cx) which have diverse functions in cell differentiation, proliferation and death via a GJ dependent or independent mechanism. Cx are also known to have diverse effects in tumor biology. However, its role in early mammary oncogensis has not been well studied.

Design: Paraffin sections of 39 DCIS were immunostained with antibodies to Cx43 (CXN-6, 1:250, Santa Cruz, CA) and Cx26 (rabbit polyclonal, 1:100, Zymed, CA). Immunoreactivity was semiquantified based on the labeling intensity (0-3) and %. A score of 0-300 was generated as the product of the intensity score and the % on each case. Cx immunoreactivity was also categorized as high or low by the median in each case and analyzed among normal, fibrocystic and neoplastic epithelia and correlated with nuclear grade, ER/PR/ Her2 status of DCIS.

Results: Normal mammary epithelium showed frequent membranous Cx26 reactivity but little Cx43. In contrast to normal epithelium, Cx43 and Cx26 reactivity tended to be cytoplasmic and was detected in 32 (86%) and 19 of 39 (49%) DCIS, respectively. Cx43 and Cx26 immunoreactivity was significantly different between DCIS and normal epithelium (Table 1).

Table 1	Normal	DCIS	Fibrocystic	
	Median/Mean	Median/Mean	Median/Mean	One-Way Anova
Cx26	210/179	3/21	100/114	p=0.0001
Cx43	0/4	50/ 82	10/21	p=0.0001

Cx26 level was marginally correlated to high nuclear grade (P=0.0509). Cx43 or Cx26 reactivity did not correlate with ER, PR or Her2 status in DCIS.

Conclusions: Cx43 is frequently over-expressed in the cytoplasm of DCIS cells. In contrast to the membrane Cx26 reactivity in normal epithelium, a smaller number of DCIS express lower level of cytoplasmic Cx26. The cytoplasmic expression of Cx43 and Cx26 is significant different between DCIS and benign epithelium. It is unclear what is the biologic mechanism for the cytoplasmic accumulation of Cx43 and Cx26 which, nevertheless, may play a role in early mammary oncogenesis. The lack of membrane localization of both Cx43 and Cx26, and the lack of correlation with ER/PR/Her2 status suggest that the functions of these two Cx in DCIS are likely GJ and ER/HER2 independent.

333 Does Nottingham Grade, MIB-1 Labeling Index (LI) Predict Recurrence Score (RS) of Oncotype Dx™ Assay?

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Background: Oncotype Dx™ assay, a RT-PCR based genomic assay analyzes the expressions of 21 genes to give a distant breast cancer RS, stratifying estrogen receptor (ER)+, node negative breast cancer patients for or against chemotherapy. In the literature, reports correlating histopathologic parameters correlating with Oncotype Dx™ RS independent of Genomic Health are limited. Here we present our experience with correlation of traditional histopathologic variables with RS.

Design: 48 cases of invasive breast cancers with Oncotype Dx data were evaluated. Clinical and morphologic findings including histologic subtype, Nottingham grade, TMN stage, ER, PR, Her2/neu status were recorded. Tissue microarray block was constructed from formalin fixed paraffin embedded tumor blocks containing duplicate, 0.6 mm cores. Immunostaining was performed using Ki-67/MIB antibody. LI was scored as percent positive tumor nuclei and were grouped into low (<25%) and high (>=25%). Statistical analysis was performed by using Chi square test and Spearman correlation. (P-value<0.05significant).

Results: Out of 48 invasive carcinomas, there were 38 ductal, 8 lobular, 1 mucinous, and 1 tubular. 17/48 (35.4%) cases were grade1, 24/48 (50%) grade 2, 7/48 (14.6%) grade 3. Distribution of MIB1 LI against grade and RS are shown in Tables1,2. RS ranged from 4-39 (mean 18) with 23/48 (47.9%) in low risk, 22/48 (45.8%) intermediate risk and 3/48 (6.3%) high risk. Overall, there was no statistical correlation between grade, MIB1 LI with RS. 5 cases had metastatic lymph nodes (4 micrometastasis): 1 high RS, grade3, low MIB-1 LI; 1 intermediate RS, grade1, high MIB1 LI and 3 low RS, grade1/2, low MIB-1LI.

Table 1			
MIB-1 LI	Grade 1	Grade 2	Grade 3
Low (n=39)	15	18	6
High (n=9)	2	6	1
Total (n=48)	17	24	7

Table 2			
MIB-1 LI	Low RS	Intermediate RS	High RS
Low (n=39)	21	15	3
High (n=9)	2	7	0
Total (n=48)	23	22	3

Conclusions: Our results confirm heterogeneity within ER+ node negative breast cancers. We identified a subset of patients having low RS with high grade (2/3) and MIB-1 LI who may benefit from additional treatment. Tumor grade and MIB1 LI complement RS score in a subset and these findings may have utility in health systems that have limitations on performing Oncotype DX due to higher cost. In cases with low MIB-1 LI and/or low grade, Oncotype DX identified high risk tumors(6 intermediate, 1 high RS) making it an independent indicator for clinical decision making. Although currently Oncotype DX is not recommended for node+ tumors, 60% of node+ cases had low RS.

334 HER2/neu Testing in Breast Cancer, a Reference Centre 8-Year Experience

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Background: Testing for the HER2/neu status became the standard of care for breast cancer management since the release of Herceptin in adjuvant and metastatic settings. Sunnybrook Health Sciences Centre is a large academic hospital with a cancer centre and a reference laboratory for HER2/neu testing. We summarized our experience with HER2/neu testing of over 10,000 cases.

Design: All breast cancer cases tested for HER2/neu between 2000 and 2008 were evaluated by 2 breast pathologists and entered into a database. Pertinent pathological characteristics including histological type, tumor grade, and percentage of ER and PR positive cells were retrieved from the pathology reports.

Results: We identified 10,149 immunohistochemical (IHC) and 1143 in-situ hybridization (ISH) tests from 9556 patients. Seventy percent of the cases were referred in. The number of cases tested increased over the audit period and varied from 460 cases in 2000 to 2528 in 2008. The proportion of HER2/neu positive ranged from 15.3-19.5% despite several changes in antibodies and scoring criteria used in the evaluation over the years. Among ductal carcinoma of no special type (n=8062), 55% were ER/PR positive and Her2 negative, 9.7% were positive for only one hormone receptor and negative for HER2/neu, 6.5% were triple positive (ER/PR/Her2 positive), 9% were ER/PR negative and HER2/neu positive, while 2.5% were positive for only one hormone receptor and positive for HER2/neu. 17.2% were triple negative (ER/PR/Her2 negative). HER2/neu was positive in only 1 of 347 classic lobular carcinomas,

and in 24/207 pleomorphic lobular carcinomas (11.6%). All 83 tubular carcinomas were ER positive and HER2/neu negative. HER2/neu was positive in 2/129 mucinous carcinomas; these tumors had grade 2 nuclei. HER2/neu positivity correlated with high tumour grade; 1.98% in low grade tumours vs. 11.69% in intermediate grade and 20% in high-grade tumours (p<0.001).

Conclusions: Our results confirm that the rate of HER2/neu positivity is less than 20%. There is strong correlation with tumour grade. Special histologic types associated with low tumour grade and positive hormonal status are almost always negative for Her2/neu over-expression. Clinical follow-up of this large cohort will be added to further enhance the value of this database; as well the large number of cases allows for an opportunity to examine molecular markers in this cohort, particularly in special histologic types.
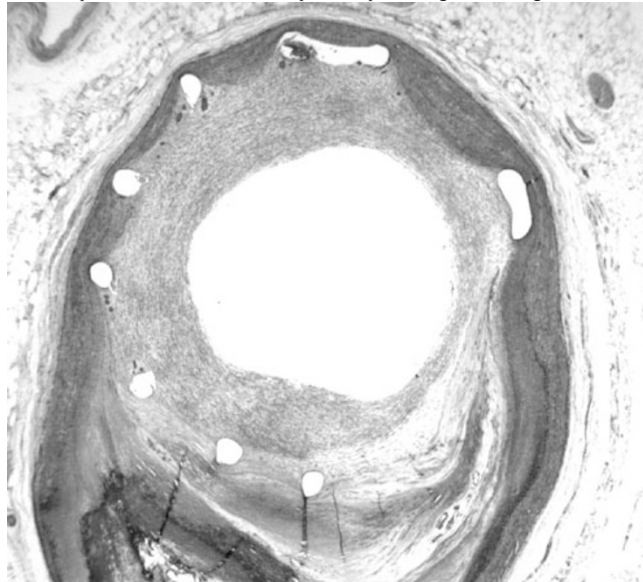
Cardiovascular

335 Electrolytic Method for Processing Coronary Arteries Containing Stents
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Background: Due to its hardness, sectioning through a stent using conventional methods causes significant damage to and/or loss of native morphology of the underlying tissue. Current specialized methods exist for making thick and thin sections through metal implants, however they are expensive, time consuming, and require a high degree of operator skill. In addition, cutting artifacts and undesirably thick microscopic sections are common. A novel electrochemical method is described and tested, which addresses these difficulties.
Design: A positive voltage was attached to a stent imbedded in formalin fixed tissue, which was then suspended in a grounded electrolytic solution. The stent dissolved over a time interval of 5 to 30 minutes, after which the tissue was sectioned. Residual small metallic fragments were removed as required. The resulting sections were processed according to standard histological techniques.
Results: Sixteen stents were dissolved using this electrolytic process. These included 316L stainless steel and Cobalt-Chromium core materials, as well as drug eluting and bare metal stents. The underlying tissue was preserved, and histological sections obtained, including H&E, Movat (figure 1), and several immunohistochemical stains. The sections were compared to those obtained from previous processing methodologies.

Conclusions: The electrolytic stent removal process was applied successfully to a broad range of stents, representing the majority of stent designs encountered in practice. Histological sections obtained compared favorably to those obtained with previous processing methodologies. Other improvements over previous methods include low cost, short processing time, short operator time, low skill level required, increased consistency of results, and compact size.

336 Arrhythmogenic Cardiomyopathy: A Biventricular Disease with Predilection for African-Americans
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Background: The ventricular distribution of arrhythmogenic cardiomyopathy (AC) in sudden death has not been studied in detail, especially in relation to racial and exertional status. There have been few immunohistochemical studies of sarcomeric related proteins.
Design: Fifty cases of sudden cardiac death with the diagnosis of AC were retrospectively studied. Distribution of disease as determined grossly and microscopically was correlated with activity at time of death, race, and presence of inflammation. AC was defined as subepicardial or right ventricular fibrofatty change surrounding altered cardiac myocytes with disordered myofilaments and vacuolated cytoplasm. Racial and gender incidence was compared to 500 cases of sudden cardiac death due to other causes seen in

consultation during the same time period. Immunohistochemical stains for connexin-43, desmin, alpha tubulin, sarcomeric actin were performed on AC cases.
Results: There were 23 whites (44%), 25 blacks (50%), and 2 Asians (6%) with AC; the proportion of blacks was greater than the non-AC sudden deaths (50% vs. 34%, p=.01). Death was exertional in 29 cases (58%) vs. 5% for non-AC SD (p<.0001) and there were 7 women (14%) vs. 26% for non-AC sudden death (p=.05). Extent of disease was predominantly right ventricular in 6 (12%, age 25±5 years), biventricular in 25 (50%, age 36±3 years), and left ventricular (ALVC) in 19 (38%, age 37±3 years), with some overlap in 38 (76%). There was no difference in proportion of blacks by ventricular distribution (p>.9). RV dilatation was present in 22 (44%) and aneurysms were present in 2 (4%); there was no correlation between RV dilatation and race or exertion. The proportion of exertional deaths was greatest in right ventricular AC (ARVC, 83%) followed by ALVC (58%) and biventricular (50%, p=0.2). Inflammation was present in 44% of biventricular AC, vs. 74% of LVNC and 83% of ARVC (p=.05). Immunohistochemical staining for sarcomeric proteins (sarcomeric actin), desmin, alpha tubulin and connexin-43 demonstrated disruption of myofilaments in areas of scarring, but no abnormalities in areas remote from fibrofatty or inflammatory infiltrates.
Conclusions: Arrhythmogenic cardiomyopathy, when presenting with sudden death, is usually biventricular, with inflammation more predominant in RV involvement. There may be a predilection for African-Americans. Sarcomeric structure appears normal in non-involved areas.

337 Molecular Autopsy of Sudden Cardiac Death: Preliminary Experience of the Northeast Italy Juvenile Sudden Death Registry
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Background: Molecular genetic screening is currently employed in the clinical diagnostic track of inherited cardiovascular diseases to identify disease-causing mutations. More recently, these techniques have been also applied to sudden death (SD) autopsies which remain unexplained after a thorough post-mortem investigation or in those with inherited cardiomyopathies. The aim of this study was to perform a genetic screening of known disease-causing genes involved in catecholaminergic polymorphic ventricular tachycardia (CPVT) or arrhythmogenic right ventricular cardiomyopathy (ARVC).
Design: Ten cases of juvenile SD (all males, age range 16-35 yrs) from the Veneto Region Registry were investigated. Genetic screening was performed for cardiac ryanodine receptor (RyR2) and calsequestrin (CASQ2) genes in two cases with structural normal heart, and for plakophilin-2 (PKP2), desmoplakin (DSP), desmoglein-2 (DSG2), desmocollin-2 (DSC2) and plakoglobin (JUP) genes in eight cases with ARVC. In five cases the investigation was conducted directly in the proband who died suddenly on frozen autoptic EDTA-blood (3), on frozen tissue (1) or on paraffin-embedded tissue (1). The other five cases were studied indirectly by screening EDTA-blood samples of parents.
Results: The autoptic paraffin embedded tissue was inadequate, whereas frozen autoptic EDTA- blood and frozen tissue were suitable for genetic investigation. Pathogenic gene mutations were identified in five SD cases: in 2 cases in the autopsy probands (one ARVC–DSG2-H790Y, one unexplained SD with structurally normal heart–RyR2-A2387P), and in the other 3 cases indirectly in the parents (all ARVC: PKP2-G59X, DSP-c.1686+1 C>T, DSG2-V56M). The mutation identified indirectly was discovered in the blood of the mother in two cases and of the father in one. None of these mutation was detected in a 100 genomics DNA (200 chromosomes) from unrelated healthy control subjects from Venetian population.
Conclusions: Molecular autopsy is mandatory on SD cases with structural normal heart as well as in those with inherited structural cardiomyopathies, since it can be potentially life-saving in terms of management and prevention of those left behind. Moreover, it is mandatory that the standard SD autopsy includes archiving either EDTA-preserved blood or frozen tissue to allow postmortem genetic testing.

338 Vascular Fibrosis Correlates with Hypertension, Kidney Function, and Diabetes in a Wide Range of Vascular Tissues
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Background: Chronic disease states are thought to affect vascular fibrosis in atherosclerosis-prone, large caliber blood vessels. We compared the degree of fibrosis in these vessels to fibrosis in other medium-sized atherosclerosis resistant vessels from the same subjects to determine if vascular fibrosis is a global phenomenon.
Design: Eight unique blood vessels (carotid, coronary, dorsalis pedis, iliac, internal mammary, mesenteric, pulmonary and renal arteries) were harvested from 100 subjects undergoing autopsy, generating 17 vascular tissue microarrays (TMAs). Slides cut from TMA blocks were stained with Masson's trichrome, and automated image analysis methods were used to quantify fibrosis in these vessels. Clinical and sociodemographic variables from the subjects were evaluated relative to the amount of fibrosis present in the tunica media and tunica intima using correlation and t-tests.
Results: In 7 of the 8 studied vessels, the percentage of fibrosis in the tunica media was associated with a clinical history of hypertension (overall $p < 0.001$, t-test). Only the pulmonary artery, which is not subject to systemic pressures, did not associate with hypertension. Also, for any given subject, poor renal function (estimated glomerular filtration rate < 30 ml/min/1.73 m²; $p < 0.001$) and a history of diabetes ($p = 0.008$) was associated with an overall increase in medial fibrosis. The age, sex, ethnicity, and smoking history of a subject showed no correlation with the degree of fibrosis in any vessel. Media tunica fibrosis was strongly correlated among all vessels within a given subject ($r = 0.24$ to 0.72; $p < 0.03$).
Conclusions: This study is the first to compare the extent of fibrosis in eight distinct blood vessels collected from the same subjects. We confirmed that hypertension, attenuated kidney function and diabetes are risk factors for medial fibrosis in atherosclerosis-prone vessels, and identified new associations in smaller atherosclerosis-resistant vessels. We