Breast Cancer Markers

PhenoPath Laboratories’ pathologists have world-class expertise in breast cancer, publishing widely in peer-reviewed journals, and speaking at national and international pathology conferences. Our pathologists have extensive experience interpreting breast cancer cases, including histopathologic analysis with integration of immunophenotypic and genotypic analyses.

Prognostic and Predictive Markers in Breast Cancer: Immunophenotypic and Genotypic Analyses

The role of the pathologist in the evaluation of breast cancer now transcends that of determining the correct morphologic diagnosis, including the grading and staging of the cancer. Breast oncologists increasingly make treatment decisions based upon the phenotypic and/or genotypic characteristics of the tumor, such as the presence of hormone receptors and the HER2 oncogene status. The most clinically relevant prognostic and predictive (i.e., treatment-guiding) markers include the following:

ER/PR: Estrogen and progesterone receptor status of a primary breast tumor is a weak prognostic marker, but a powerful predictive marker of response to tamoxifen and other hormonal therapies. ER and PR determination by IHC in the setting of formalin-fixed, deparaffinized tissue remains the most reliable method to determine hormone receptor status in breast cancer. Moreover, PhenoPath Laboratories employs state-of-the-art rabbit monoclonal antibodies (SP1) demonstrated in a long-term follow-up study to better predict outcome and response to tamoxifen than the mouse monoclonal antibody (1D5) and polymer detection systems that have been clinically validated in large, tissue microarray-based studies with long-term clinical follow-up. In accordance with ASCO-CAP Guidelines, PhenoPath tracks the proportion of cases positive for ER and PR in women and has met the required standards that the proportion of cases that are ER negative does not exceed 30% for women under the age of 65 and 20% for women over the age of 65. PhenoPath is one of very few national laboratories listed by CAP that have validated ER and PR assays and offer reference testing for antibody validation.


HER2: Amplification of the HER2 gene and attendant protein overexpression are present in 10% to 20% of primary breast cancers. HER2 overexpression and/or gene amplification is a negative overall prognostic marker, as confirmed in gene array studies. HER2 status also predicts sensitivity to anthracycline-based chemotherapy regimens, as well as relative resistance to cytoxan-based regimens and tamoxifen-based therapies in the setting of estrogen receptor-positive breast cancers. Most importantly, breast cancers with HER2 alterations are now treated with specific anti-HER2 targeted therapies which have been shown to markedly improve response rate and survival when added to chemotherapy or as monotherapy.

Detection of these alterations can be studied either by IHC, looking for protein overexpression, or fluorescence in situ hybridization (FISH), looking for gene amplification. In normal breast epithelium and breast cancers without HER2 alterations, techniques such as FISH detect two HER2 signals - one on each copy of chromosome 17, and IHC shows low or absent signal representing HER2 protein expression. In the breast cancers showing HER2 alterations, gene amplification is invariably present, with increased HER2 signals, and IHC almost always showing a strong membranous (3+) pattern of expression. According to the ASCO-CAP Guidelines, when simultaneously looking at FISH probes to both the HER2 and the chromosome 17 centromeric region, the ratio of HER2/CEP17 ratio of 1.8 is considered negative, between 1.8 and 2.2 is considered equivocal, and >2.2 is considered positive for HER2 amplification. At PhenoPath, FISH preparations are scored and analyzed by pathologists, which ensures accurate identification of the tumor cell population. Furthermore, PhenoPath Laboratories employs the MetaSystems image analysis system, which greatly increases the throughput and permits more comprehensive analysis of the tumor, counting hundreds or even thousands of tumor cells, rather than the minimum 20 cells analyzed in other laboratories.

In accordance with the ASCO-CAP Guidelines, we recommend the use of IHC as an initial screen for HER2 status, with FISH studies performed on cases that show 2+ levels of immunostaining; such cases are indicated as showing an ‘equivocal’ HER2 status by IHC.

PhenoPath has an extensive quality assurance program that monitors the high concordance rate of HER2 IHC and FISH on a quarterly basis to assure accuracy in the assessment of HER2 status in breast cancers. In addition, in order to meet ASCO-CAP HER2 testing requirements, PhenoPath has established a program to validate HER2 tests performed in other laboratories in order to compare the results to our FISH testing.
HER2 TESTING CONCORDANCE

Amplification of the HER2 gene and concomitant protein overexpression are both present in 10% to 20% of primary breast cancers, and identification of this subset of breast cancers is key. The ASCO-CAP Guidelines, issued in 2007, require laboratories to demonstrate at least 90% concordance between results of HER2 status by different methods (e.g., IHC and FISH); there have been very few publications documenting this high level of concordance. However, pathologists at Phenopath Laboratories have published several studies documenting the high level of accuracy of HER2 IHC and FISH when compared with other methodologies. In a 2008 study of 6,604 breast cancer specimens, among HER2-negative cases (as determined by IHC), the concordance with FISH was 99.2%; among the HER2-positive cases (as determined by IHC), the concordance rate was 94.7%. In a 2010 study, comparing HER2 FISH at Phenopath with HER2 RT-PCR results performed at Genomic Health, Inc., a positive concordance rate of 98% and a negative concordance rate of 97% was found. These results reflect the many years of experience of our pathologists and staff, our extensive and demanding ongoing quality assurance program for HER2 testing, and the overall dedication to quality testing at Phenopath Laboratories.


DOES POLYSOMY 17 EXIST IN BREAST CANCER?

According to the ASCO-CAP guidelines, when the HER2/CEP17 ratio exceeds 2.2, the tumor is classified as amplified. However, when both HER2 and CEP17 signals are increased in tandem, it has been assumed that this represents polyplody of chromosome 17, and if the ratio is below 1.8, such patients are not considered candidates for HER2-targeted therapy.

In a paper published by the pathologist scientists at Phenopath Laboratories, in a series of specimens from patients whose breast cancers were shown by FISH testing to have elevated copy numbers of both HER2 and CEP17, FISH studies examining the status of three other genes on chromosome 17 (SMS, RARA, and p53) were performed. Nearly half (43.9%) of patients initially classified as negative for HER2 amplification owing to the presence of ‘polyomy 17’ were reclassified as amplified.

These findings suggest that in many, or perhaps most cases thought to demonstrate ‘polyomy 17’, the increased CEP17 and HER2 signals result not from polyomy but from the presence of an amplicron that spans part of the chromosome 17 that includes both HER2 and CEP17.

The demonstration of what appears to be true amplification of the HER2 gene in a subset of patients previously thought to be negative could alter treatment decisions for many breast cancer patients.

In general, the Ki-67-defined cell proliferation index correlates with histologic grade and is a prognostic marker of clinical outcome. Ki-67, along with other markers of cell proliferation, are major components measured in the Oncotype DX assay. IHC studies can be used to quantify cell proliferation in breast cancers by using MIB-1, a ‘second generation’ antibody to the Ki-67 antigen, a protein complex expressed during all non-G0 (i.e., non-resting) phases of the cycle. Data suggest that a Ki-67 defined cell proliferation index above 10-14% defines a high-risk group in terms of prognosis. Furthermore, studies also indicate that determination of the Ki-67 index might have a valuable role in predicting benefit from specific treatments in subtypes of breast cancer.

Basal-Like Breast Cancer

Gene expression profiling studies, employing analyses of hundreds of different genes simultaneously, have demonstrated that breast cancer can be subdivided into several distinct subtypes (luminal A, luminal B, HER2 overexpressing, basal-like, and normal breast-like) based on patterns of gene expression. These molecularly defined tumor subtypes have been shown to have distinctly different clinical outcomes and their identification may play an increasingly important role in guiding therapy in breast cancer. The subtyping may be partially recapitulated by IHC using a limited panel of markers (including ER, PR, and HER2). Molecular gene expression studies have demonstrated that approximately 15-20% of breast cancers correspond to the basal-like subtype. Identification of this subset is important, as patients with this subtype have a particularly poor clinical outcome, and these tumors represent ‘triple negatives’ (i.e., are ER- and PR-negative and negative for overexpression of HER2) and are not candidates for many conventional therapies. Tumors of the basal-like subset have also been demonstrated to possess a unique immunophenotype as assessed in standard, formalin-fixed tissue sections, with expression of cytokeratin 5, p63, EGFR, c-kit, and overexpression of p53 in a significant fraction of cases. Basal-like breast cancers are more prevalent in the premenopausal setting and in patients of African-American ancestry. Patients with inherited BRCA1 mutations are also more likely to have the basal-like breast cancer subtype.

Myoepithelial Markers

Normal breast ducts and lobules are comprised of two epithelial cell layers. Loss of the outer myoepithelial layer is a hallmark of infiltrating carcinoma in the breast. The outer myoepithelial layer is retained in all benign proliferative processes as well as ductal carcinoma in situ. Consequently, identification of the presence or loss of myoepithelium using antibodies to myoepithelial-specific proteins can help in a number of important problems in breast pathology, including: (a) distinguishing in situ vs. infiltrating carcinoma, e.g., ruling in or out the presence of microinvasion; (b) distinguishing sclerosing adenosis and other benign sclerosing lesions from infiltrating carcinoma; (c) distinguishing benign papillomas from papillary carcinoma, and others. These studies are well-suited to core needle biopsies, where low-power architecture is often difficult to appreciate. Published studies performed at PhenoPath Laboratories and elsewhere point to smooth muscle myosin heavy chain (SMMHC) and p63 as the most sensitive and specific markers of myoepithelium for this purpose, although other markers (cytokeratin 5, CD10, calponin, etc.) can be of help in some cases.

Lobular vs. Ductal Carcinoma of the Breast

It has been demonstrated that in histologic settings where ductal and lobular neoplasia may be confused (and particularly in the setting of in situ disease, where there can be significant differences in patient management), loss of expression of E-cadherin by IHC can confirm the diagnosis of lobular carcinoma, even in the setting of non-classical morphologic findings. In lobular neoplasia, mutation or silencing of the E-cadherin gene results in loss of expression of E-cadherin, a cell surface adhesion molecule present in normal breast epithelium, as well as ductal carcinoma. Owing to the role of E-cadherin in ‘homotypic’ cell to cell binding, loss of expression of this protein probably accounts for the characteristic non-cohesive ‘single cell’ growth pattern of infiltrating lobular carcinoma.

This infiltrating ductal carcinoma of the breast in a 40-year-old female shows an H&E pattern that could represent either ductal carcinoma in situ or infiltrating cribriform carcinoma. Antibodies to the myoepithelial-specific proteins, smooth muscle myosin heavy chain (SMMHC) and p63 show retention of the outer myoepithelial layer around all tumor cell profiles, pointing to the diagnosis of ductal carcinoma in situ. Note the nuclear signal of p63 in contrast to the cytoplasmic signal of SMMHC.

This biopsy from a 40-year-old female shows an H&E pattern that could represent either ductal carcinoma in situ or infiltrating cribriform carcinoma. Antibodies to the myoepithelial-specific proteins, smooth muscle myosin heavy chain (SMMHC) and p63 show retention of the outer myoepithelial layer around all tumor cell profiles, pointing to the diagnosis of ductal carcinoma in situ. Note the nuclear signal of p63 in contrast to the cytoplasmic signal of SMMHC.

Basal-like breast cancer showing low (left) and high (right) Ki-67-defined cell proliferation indices using the MIB1 antibody.