Breast Cancer Assays of Genetic Expression in Tumor Tissue

I. Description

Laboratory tests have been developed that detect the expression, via messenger RNA (mRNA) or protein, of many different genes in breast tumor tissue and combine the results into a prediction of distant recurrence risk for women with early stage breast cancer. Test results may help providers and patients decide whether to include adjuvant chemotherapy in post-surgical management.

21-gene Recurrence Score (Oncotype DX):

The assay is supported by strong evidence of clinical validity, i.e., that the recurrence score (RS) is strongly associated with risk of distant recurrence in women with invasive breast cancer that is positive for hormone receptors, negative for HER2, and without lymph node involvement. Limited but sufficient evidence supports analytic validity and clinical utility in this population. Oncotype DX adds additional risk information to the conventional clinical classification of individual high-risk patients and identifies a subset of patients who would otherwise be recommended for chemotherapy but who are actually at lower risk of recurrence (average 7–9% risk at 10 years; upper 95% confidence interval (CI) limits: 11–15%). Thus, a woman who prefers to avoid the toxicity and inconvenience of chemotherapy and whose Oncotype DX RS value shows that she is at very low risk of recurrence might reasonably decline chemotherapy.

In similar women who are node-positive, the evidence is less clear that the risk of recurrence in low-risk RS patients is sufficiently low or that the benefit of chemotherapy is sufficiently large, to recommend avoiding otherwise currently recommended treatment. Additional studies are necessary and ongoing. Currently available evidence is insufficient to determine that Oncotype DX improves the net health outcome in women with hormone receptor–positive, HER2-negative, lymph node–positive invasive breast cancer. Evidence for a significant incremental improvement in outcomes when Oncotype DX is added to conventional clinical risk classifiers is lacking.

For women with ductal carcinoma in situ (DCIS), the use of a subset of genes from the 21-gene recurrence score (i.e., Oncotype DX DCIS) to predict recurrence and inform treatment planning post-excision, development and clinical validity studies have not yet been published to allow full
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... evaluation. Moreover, no information is yet available on whether women are better categorized as to their recurrence risk by the Oncotype DX DCIS Score compared with standard clinical indicators.

**70-gene signature (MammaPrint):**
A large number of studies of clinical validity, and a few attempting to address the clinical utility of the 70-gene signature have been published. Several studies have pooled and re-analyzed subsets of previously published data in attempts to arrive at more homogeneous sample populations. Nevertheless, the studies of the 70-gene signature continue to suffer from confounding in heterogeneous sample populations. Pooled re-analyses of subpopulations may control for one variable (e.g., nodal status), but confounding remains from other variables (e.g., treatment heterogeneity). Results for the 70-gene signature good prognosis patients have confidence intervals that extend into ranges that likely confer too much risk for patients and providers in the U.S. Because the test result is not a continuous numerical result, patients cannot view their result within the spectrum of good prognosis results and adjust their preferences accordingly. The recently presented Microarray Prognostics in Breast Cancer (RASTER) study represents an improved study design, and results suggest that MammaPrint may accurately re-classify early, node-negative invasive breast cancer patients classified high risk for recurrence by clinical and pathologic variables to low risk, such that chemotherapy may not be necessary. However, the study is not yet published, patient numbers and events are too low for firm conclusions, and follow-up is not yet sufficiently mature. Currently available evidence is insufficient to determine that MammaPrint® improves the net health outcome in women with early-stage, invasive breast cancer. Although evidence suggests that there may be incremental improvement in risk classification when MammaPrint® is added to conventional clinical risk classifiers, the quality of the evidence is insufficient to draw firm conclusions.

**Intrinsic Subtype Classifiers BluePrint and TargetPrint:**
The 80-gene expression assay BluePrint discriminates among 3 breast cancer molecular subtypes, and TargetPrint is a method to measure ER, PR, and HER2 as an alternative to immunohistochemistry and FISH. Clinical utility of BluePrint is unknown, as it is unclear how this test will add to treatment decision making using currently available, accepted methods (eg, clinical and pathologic parameters). The incremental benefit of using TargetPrint as an alternative to current standard methods of measuring ER, PR, and HER2 has not been demonstrated, nor is it included in recommendations for testing issued by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists.

**Other Gene Expression Profiling Tests Mammostrat Breast Cancer Test, Breast Cancer Index, BreastOncPx, and 4-Protein Immunohistochemical Score (NexCourse Breast IHC4), Breast PRS, Prosigna, EndoPredict:**
The available evidence supporting these tests consists of clinical validity data showing that the test is independently and significantly associated with distant recurrence and that the test can identify a lower risk population of women with early, invasive breast cancer who may not need chemotherapy. In almost all cases, the test is not added to and compared with a standard clinicopathologic classifier such as Adjuvant! Online. The BreastOncPx validation study included a receiver operating characteristic (ROC) analysis comparing the test with Adjuvant! Online, but no clear evidence supporting clinical utility was available. NexCourse Breast IHC4
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(immunohistochemical markers) was compared with standard clinicopathological prognostic classifiers in a reclassification analysis and was shown to accurately reclassify significant numbers of patients from high and intermediate risk to low risk, but numbers in the study were small and insufficient for conclusions. Studies that evaluated Prosigna in combination with clinical factors either did not provide a reclassification analysis (Dowsett et al, 2013) or used cut points that differed from the marketed test (Gnant et al, 2015). Available evidence is therefore insufficient to determine that the Breast Cancer IndexSM, Mammostrat Breast Cancer Test, BreastOncPx, NexCourse Breast IHC4, Prosigna, BreastPRS, or EndoPredict improve the net health outcome in women with early-stage, invasive breast cancer. Evidence for a significant incremental improvement in outcomes when each test is added to standard risk classification based on clinical and pathologic parameters is lacking.

Table 1. Gene Expression Tests for Breast Cancer Commercially Available in the U.S.

<table>
<thead>
<tr>
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<tr>
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<td>Mammaprint</td>
<td>Agendia</td>
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ER: Estrogen receptor; HER2: human epidermal growth factor receptor 2; IHC: immunohistochemistry; Ki-67: a marker of of tumor proliferation; MGI: Molecular Grade Index; PAM50: Prediction analysis of microarray 50 gene set; PR: Progesterone receptor; RT-PCR: Real-time reverse transcriptase-polymerase chain reaction

**BluePrint and TargetPrint**

Gene expression patterns have led to the identification of molecular subtypes of breast cancer, which have different prognoses and responses to treatment regimens. These molecular subtypes are largely distinguished by differential expression of estrogen receptors ER, progesterone receptors (PR), and human epidermal growth factor receptor 2 (HER2) in the tumor, and are classified as luminal, basal, or HER2 type. Luminal type breast cancers are ER-positive; basal type breast cancers correlate best with ER-, PR-, and HER2-negative (“triple negative”) tumors, and HER2 type, with high expression of HER2.
II. Criteria/Guidelines

A. The use of the 21-gene reverse transcriptase-polymerase chain reaction (RT-PCR) assay (i.e. Oncotype DX) to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy is covered (subject to Limitations/Exclusions and Administrative Guidelines) in women with primary, invasive breast cancer meeting all of the following characteristics:
1. Unilateral tumor;
2. Hormone receptor positive (that is, estrogen-receptor [ER]-positive or progesterone-receptor [PR]-positive);
3. Human epidermal growth factor receptor 2 (HER2) negative; (See Appendix for definitions)
4. Tumor size is 0.6 to 1 cm with moderate/poor differentiation or unfavorable features (angiolympathic invasion, high histologic grade or high nuclear grade) OR tumor size larger than 1 cm;
5. Node negative (lymph nodes with micro-metastases [less than 2 mm in size] are considered node negative);
6. Who will be treated with adjuvant endocrine therapy (e.g., tamoxifen or aromatase inhibitors).
7. When the test result will aid the patient in making the decision regarding chemotherapy (i.e., when chemotherapy is a therapeutic option); AND
8. When ordered within 6 months following diagnosis, since the value of the test for making decisions regarding delayed chemotherapy is unknown.

B. The 21-gene RT-PCR assay Oncotype DX should only be ordered on a tissue specimen obtained during surgical removal of the tumor and after subsequent pathology examination of the tumor has been completed and determined to meet the above criteria (i.e., the test should not be ordered on a preliminary core biopsy). The test should be ordered in the context of a physician-patient discussion regarding risk preferences when the test result will aid in making decisions regarding chemotherapy.

C. For patients who otherwise meet the above characteristics but who have multiple ipsilateral primary tumors, a specimen from the tumor with the most aggressive histological characteristics should be submitted for testing. It is not necessary to conduct testing on each tumor; treatment is based on the most aggressive lesion.

III. Limitations/Exclusions

A. All other indications for the 21-gene RT-PCR assay (i.e., Oncotype DX), including determination of recurrence risk in invasive breast cancer patients with positive lymph nodes or patients with bilateral disease, is not covered as it is not known to improve health outcomes.

B. Use of a subset of genes from the 21-gene RT-PCR assay for predicting recurrence risk in patients with noninvasive ductal carcinoma in situ (i.e., Oncotype DX DCIS) to inform treatment planning following excisional surgery is not covered as it is not known to improve health outcomes.
C. The use of other gene expression assays (e.g., MammaPrint 70-gene signature, Mammostrat Breast Cancer Test, the Breast Cancer Index, the BreastOncPx, NexCourse Breast IHC4, BreastPRS, Prosigna and EndoPredict for any indication are not covered as they are not known to improve health outcomes.

D. The use of gene expression assays to molecularly subclassify breast cancer (e.g, BluePrint) are not covered as they are not known to improve health outcomes.

E. The use of gene expression assays in men with breast cancer is not covered as it is not known to improve health outcomes.

F. The use of gene expression assays for quantitative assessment of ER, PR, and HER2 overexpression (eg, TargetPrint) are not covered as they are not known to improve health outcomes.

IV. Administrative Guidelines

A. Precertification is not required.

B. Documentation supporting the medical necessity should be legible, maintained in the patient's medical record and must be made available to HMSA upon request. HMSA reserves the right to perform retrospective review using the above criteria to validate if services rendered met payment determination criteria.

C. Applicable codes:

<table>
<thead>
<tr>
<th>CPT</th>
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<tbody>
<tr>
<td>81519</td>
<td>Oncology (breast), mrna, gene expression profiling by real-time rt-pcr of 21 genes, utilizing formalin-fixed paraffin embedded tissue, algorithm reported as recurrence score</td>
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<tr>
<td>S3854</td>
<td>Gene expression profiling panel for use in the management of breast cancer treatment</td>
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V. Background

For women with early-stage, invasive breast cancer (i.e. cancer extends beyond the basement membrane of the milk ducts into adjacent tissue), adjuvant chemotherapy provides the same proportional benefit regardless of prognosis. However, the absolute benefit of chemotherapy depends on the baseline risk of recurrence. For example, women with the best prognosis have small tumors, are estrogen-receptor-positive, and lymph node negative. These women have an approximately 15% baseline risk of recurrence; approximately 85% of these patients would be disease-free at 10 years with tamoxifen treatment alone and could avoid the toxicity of chemotherapy, if they could be accurately identified. Conventional risk classifiers estimate recurrence risk by considering criteria such as tumor size, type, grade, and histologic characteristics; hormone receptor status; and lymph node status. However, no single classifier is considered a gold standard, and several common criteria have qualitative or subjective components that add variability to risk estimates. As a result, more patients are treated with
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chemotherapy than can benefit. Better predictors of baseline risk could help women, who prefer to avoid chemotherapy if assured that their risk is low, make better treatment decisions in consultation with their physicians.

Recently, several groups have identified panels of gene expression markers (“signatures”) that appear to predict the baseline risk of invasive breast cancer recurrence after surgery, radiation therapy, and endocrine therapy (for hormone-receptor-positive tumors). Several gene expression tests are commercially available in the U.S. are listed in Table 1. If these panels are more accurate than current conventional classifiers, they could be used to aid chemotherapy decision making, when current guidelines do not strongly advocate its use, without negatively affecting disease-free and overall survival (OS).

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At present, methodology for molecular subtyping is not standardized, and breast cancer subtyping is routinely assessed by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH).

BluePrint is an 80-gene expression assay that classifies breast cancer into basal type, luminal type or HER2-type. The test is marketed as an additional stratifier into a molecular subtype after risk assessment with MammaPrint.

TargetPrint is a microarray-based gene expression test that offers a quantitative assessment of ER, PR, and HER2 overexpression in breast cancer. The test is marketed to be used in conjunction with MammaPrint and BluePrint.

FDA Status

MammaPrint was U.S. Food and Drug Association (FDA)-approved on February 6, 2007. MammaPrint is performed in Agendia laboratories in the Netherlands and in California. On January 23, 2015, MammaPrint received FDA 510(k) marketing clearance for use in fresh-frozen, paraffin-embedded breast cancer tissue.

Prosigna received 510(k) clearance from FDA based on substantial equivalence to MammaPrint on September 6, 2013.

Other tests mentioned in this policy are offered as laboratory-developed tests under Clinical Laboratory Improvement Amendments (CLIA) –licensed laboratories. Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratories offering such tests as a clinical service must meet general regulatory standards of CLIA and must be licensed by CLIA for high complexity testing.

VI. Rationale

In 2014, a TEC Assessment addressed gene expression profiling in women with lymph node-negative breast cancer to select adjuvant chemotherapy, specifically use of Oncotype DX, MammaPrint, the Breast Cancer IndexSM, and Prosigna/PAM50 gene expression assay. The Assessment concluded that use of Oncotype DX to determine recurrence risk for deciding whether to undergo adjuvant chemotherapy in women with unilateral, hormone receptor-positive, lymph node-negative breast cancer who will receive hormonal therapy meets the Blue Cross and Blue Shield Association Technology Evaluation Center (TEC) criteria; and that use of MammaPrint, the Breast Cancer IndexSM, and Prosigna to determine recurrence risk in women with unilateral,
hormone receptor-positive, lymph node-negative breast cancer who will receive hormonal therapy does not meet TEC criteria.

**Oncotype DX**

**Description**

The initial indications for the 21-gene expression profile (Oncotype DX) were newly diagnosed invasive breast cancer patients with stage I or II disease that is node-negative and estrogen-receptor (ER)-positive, who would be treated with tamoxifen. Primary validation studies enrolled node-negative patients; this indication is reviewed first. More recently, Genomic Health has expanded their indication to include all stage II disease (tumor <2 cm with spread to axillary lymph nodes or 2-5 cm without lymph node involvement); and ductal carcinoma in situ (DCIS); these indications are reviewed separately.

Results from the Oncotype DX 21-gene expression profile are combined into a recurrence score (RS). Based on a study of analytic validity, tissue sampling rather than technical performance of the assay is likely to be the greatest source of variability in results. The 21-gene expression profile was validated in studies using archived tumor samples from subsets of patients enrolled in already completed randomized controlled trials (RCTs) of early breast cancer treatment. Patients enrolled in the trial arms from which specimens were obtained had primary, unilateral breast cancer with no history of prior cancer and were treated with tamoxifen; tumors were ER-positive, most were human epidermal growth factor receptor 2 (HER2)-negative, and in the case of at least 1 trial, multifocal tumors were excluded.

**Lymph Node-negative Patients**

Studies delineating the association between the 21-gene RS and recurrence risk are shown in Table 2. Results indicate strong, independent associations between the RS and distant disease recurrence or death from breast cancer. In secondary reclassification analyses of the Paik et al. data, patient risk levels were individually classified by conventional risk classifiers, then re-classified by Oncotype DX. Oncotype DX adds additional risk information to the conventional clinical classification of individual high-risk patients and identifies a subset of patients who would otherwise be recommended for chemotherapy but who are actually at lower risk of recurrence (average 7–9% risk at 10 years; upper 95% confidence interval [CI] limits: 11–15%). The analysis does not indicate significant erroneous reclassification given known outcomes. Thus, a woman who prefers to avoid the toxicity and inconvenience of chemotherapy and whose Oncotype DX RS value shows that she is at very low risk of recurrence might reasonably decline chemotherapy. The lower the RS value, the greater the confidence the woman can have that chemotherapy will not provide net benefit; outcomes are improved by avoiding chemotherapy toxicity.

**Table 2. Summary of Oncotype DX RS and recurrence risk studies**

<table>
<thead>
<tr>
<th>Study</th>
<th>Total N</th>
<th>Study Objective</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study Type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paik et al. 2004a</td>
<td>668</td>
<td>Predict recurrence</td>
<td>RS risk     % of patients</td>
</tr>
<tr>
<td>TAM arm of</td>
<td>Low (&lt;18)</td>
<td>51</td>
<td>6.8 (4.0–9.6)</td>
</tr>
<tr>
<td>Study</td>
<td>Method</td>
<td>Patients</td>
<td>Outcome</td>
</tr>
<tr>
<td>-------</td>
<td>--------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td>NSABP B-14 RCT</td>
<td>Intermed (18–30)</td>
<td>22</td>
<td>14.3</td>
</tr>
<tr>
<td></td>
<td>High (&gt;31)</td>
<td>27</td>
<td>30.5</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>100</td>
<td>15</td>
</tr>
<tr>
<td>Paik et al. 2004b</td>
<td>Reclassification study; determine incremental risk compared to conventional classifier</td>
<td>668</td>
<td>Low (8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High (92%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Bryant 2005</td>
<td>Reclassification study; determine incremental risk compared to conventional classifier</td>
<td>668</td>
<td>Low (53%)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Int-High (47%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Int-High</td>
</tr>
</tbody>
</table>

Abbreviations: DRF, distant recurrence-free; ER, estrogen receptor; N, total number of patients; NR, not reported; RS, Oncotype DX recurrence score; K-M, Kaplan Meier; NSABP, National Surgical Adjuvant Breast and Bowel Project; RCT, randomized controlled trial; TAM, tamoxifen; NCCN, National Comprehensive Cancer Network (2004); Int/Intermed, Intermediate.

1 Percentages are percent of total N.

2 Estimated from graphs. Note that different outcomes were reported between Paik et al. 2004b and Bryant 2005 and could not be converted to similar outcomes with confidence intervals.

An additional study, in which samples from a RCT of ER-positive, node-negative breast cancer patients treated with tamoxifen versus tamoxifen plus chemotherapy were tested by Oncotype DX, provides supportive evidence. RS high-risk patients derived clear benefit from chemotherapy, whereas the average benefit for other patients was statistically not significant, although the confidence intervals were wide and included the possibility of a small benefit.

The 2014 Assessment concluded that the 21-gene RT-PCR assay Oncotype DX meets criteria for women similar to those in the validation studies, i.e. women younger than 70 years of age (or with a life expectancy greater than 10 years), with unilateral, non-fixed, ER-positive, node-negative (by
full axillary dissection) invasive carcinomas, who are treated with surgery (mastectomy or lumpectomy), radiation therapy, and tamoxifen. In 1 trial, patients in the experimental arm were also treated with cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) or myelofibrosis (MF) chemotherapy. Most (92%) patients were negative for HER2.

Because clinical care for breast cancer patients has evolved since the original trials from which archived samples were acquired for assay validation, differences in evaluation and treatment regimens were considered. It was concluded that the 21-gene Oncotype DX meets the TEC criteria for the following women with node-negative invasive breast cancer:

- Those receiving aromatase inhibitor (AI)-based endocrine therapy instead of tamoxifen therapy. AI-based therapy would likely reduce recurrence rates for all RS risk groups. Thus, if a patient declined chemotherapy today on the basis of a low-risk RS (risk categories defined by outcomes with tamoxifen treatment), the even lower risk associated with AI treatment would not change that decision. This has been confirmed in the prospectively planned and blinded analysis of samples from the completed Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trial, which evaluated 5 years of anastrozole, tamoxifen, or the combination of both in postmenopausal women with localized breast cancer. The relative risk reduction for anastrozole compared with tamoxifen was similar across different values of the RS, and the risk for distant recurrence in RS low-risk patients was as low or lower than reported in the original validation studies.

- Those receiving anthracycline-based chemotherapy instead of CMF. The type of chemotherapy does not change the interpretation of the Oncotype DX risk estimate. In addition, a recent meta-analysis indicates that anthracyclines do not improve disease-free survival (DFS) or overall survival (OS) in women with early, HER2-negative breast cancer, and therefore may not be prescribed in this population.

- Lymph nodes with micrometastases are not considered positive for purposes of treatment recommendations. Current practice largely involves a detailed histologic examination of sentinel lymph nodes, allowing for the detection of micrometastases (less than 2 mm in size).

- Those whose tumors are estrogen-receptor (ER)-positive or progesterone-receptor (PR)-positive. Only ER-positive women were enrolled in Oncotype DX validation studies, whereas current clinical guidelines include either ER or PR positivity in the treatment pathway for hormone receptor-positive women with early breast cancer. Recent studies show that ER-negative, PR-positive patients also tend to benefit from endocrine therapy. Studies documenting the low incidence (1%-4%) and instability (lack of reproducibility) of the ER-negative/PR-positive subtype (15) and the reduction in reports of this subtype with improved assay techniques suggest that this subtype may represent a false negative result.

Tzeng et al. examined how women receive and incorporate the results of Oncotype DX using mailed survey and chart review. About two thirds of women believed they understood most or all of what they were told about their recurrence risk based on their test results; the majority who experienced test-related distress had intermediate or high estimated recurrence risks by RS result. The objective, recalled, and perceived recurrence risks by women in the study were surprisingly
similar, and 95% agreed that the test gave them a better understanding of their treatment options and chances of success. However, about one third of women believed they understood only a moderate amount or less during these discussions. The study was limited in generalizability in that participants were mostly Caucasian, well-educated women who had health insurance and came from urban areas.

Several studies have been published regarding the impact of RS results on chemotherapy recommendations by medical oncologists. These studies generally reported that decisions changed in about 25-40% of patients, with physician knowledge of RS, most often from endocrine therapy plus chemotherapy to endocrine therapy alone. For example

- In a retrospective reclassification analysis, Joh et al. found that inclusion of the Oncotype DX recurrence scores resulted in a 25% change in (after the fact) treatment recommendations, resulting in fewer patients projected to receive chemotherapy.
- Hassett et al. evaluated registry data from the National Comprehensive Cancer Network (NCCN) Breast Cancer Outcomes Database Project focusing on women diagnosed for hormone-receptor (HR)-positive stage I to III unilateral breast cancer during 2006-2008. Compared to women with Oncotype DX-determined intermediate-risk cancer, women with Oncotype-determined high-risk cancers were more likely to receive chemotherapy (odds ratio [OR]: 12.0; 95% CI: 6.7 to 21.3) and women with low-risk cancers were less likely to receive chemotherapy (OR: 0.1; 95% CI: 0.1 to 0.2).
- Carlson et al. (2013) conducted a systematic review of studies of Oncotype DX used to inform actual adjuvant chemotherapy decisions in ER-positive, lymph node-negative patients with early stage breast cancer. In 8 identified studies (total N=1437), Oncotype DX® RS changed the chemotherapy recommendation based on clinical-pathologic factors in 33% of patients. Compared with Oncotype DX high risk patients, low risk patients were statistically more likely to follow Oncotype DX-directed treatment (relative risk [RR], 1.07; 95% CI, 1.01 to 1.14).

Some view these as evidence of clinical utility because more patients avoid the toxicity of chemotherapy. However, there are no actual patient outcomes attached to these studies. In addition, none of the studies formalize and describe the way in which information is delivered to the patient, nor do they evaluate how patient preferences are incorporated into the final treatment decision. Lo et al. conducted a prospective multicenter study that examined both physician and patient treatment selection, as well as the impact of the RS result on patients’ anxiety, quality of life, and satisfaction with choice of treatment. However, the study did not ensure that results were presented in a consistent formal for all patients.

**Lymph node-positive patients**

Albain et al. evaluated samples from the Southwest Oncology Group Trial 8814, in which randomized node-positive, ER-positive patients treated with tamoxifen for 5 years were compared to those treated with cyclophosphamide, doxorubicin, fluorouracil (CAF) chemotherapy followed by
tamoxifen (CAF-T) for 5 years. Samples were available for determination of RS for 41% (n=148) and 39% (n=219) of the trial arms, respectively.

In this study, 10-year disease-free survival (DFS) and overall survival (OS) outcomes in the tamoxifen study arm differed by RS risk category (p=0.017 and 0.003, respectively), indicating that the RS is prognostic. When the 2 treatment arms were compared within RS risk categories, only patients in the high RS category significantly benefited from the addition of CAF to tamoxifen (for DFS, 42% [tamoxifen] vs. 55% [CAF-T], p=0.033; for OS, 51% [tamoxifen] vs. 68% [CAF-T], p=0.027), suggesting that RS is also predictive of response to chemotherapy.

A multivariable analysis of RS interaction with DFS, adjusted for number of positive nodes, was significant for the first 5 years of follow-up at p=0.029 and remained significant after adjusting for age, race, tumor size, progesterone receptor status, grade, p53, and HER2. However, the interaction was not significant (p=0.15) after adjusting for ER level (ER gene expression is a component of the 21-gene profile). Interaction results were similar for OS.

Dowsett et al. included a separate evaluation of node-positive patients in their examination of the ATAC trial samples. Of 306 node-positive patients, 243 had 1-3 involved nodes, and 63 patients, 4 or more; these were not evaluated separately. Rates of distant recurrence at 9 years were 17% (95% CI: 12-24%), 28% (20-39%), and 49% (35-64%), respectively. It is not clear that the risk of distant recurrence in low-risk RS patients would be sufficiently low to forgo the choice of chemotherapy. The authors note that their study “did not directly evaluate the value of RS in predicting the benefit of chemotherapy.”

Goldstein et al. evaluated samples from the Eastern Cooperative Oncology Group E2197 trial, which included patients with 0-3 positive lymph nodes and operable tumor greater than 1 cm in size. Patients were randomly assigned to doxorubicin plus cyclophosphamide or docetaxel plus 5 years of endocrine therapy; but outcomes were not significantly different for the study arms. A case-control study of samples from this trial found that low-risk RS patients with 0-1 positive nodes had a recurrence risk of 3.3% (95% CI: 2.2-5%), and low-risk patients with 2-3 positive nodes had a recurrence risk of 7.9% (4.3-14.1%). RS was also a significant predictive of risk regardless of nodal status.

Brufsky (2014) reviewed the 3 studies above. The review was sponsored by Genomic Health, manufacturer of Oncotype DX®, and acknowledged the need for RxPONDER results (listed in Table 7) to confirm the findings of Albain et al (2010).

Chang et al., reported that in women with locally advanced breast cancer treated with neoadjuvant docetaxel (n=97), a complete response was more likely in those with a high RS (p=0.008). (34) Gianni et al. studied 93 patients with locally advanced breast cancer who received neoadjuvant taxane chemotherapy, then post-surgery CMF treatment and tamoxifen (if ER-positive). (30) The authors reported that pathological complete response was more likely in patients with high RS results than with low RS results (p<0.01).

One study surveyed oncologists ordering the 21-gene profile for lymph node-positive patients to determine the effect of the assay results on treatment recommendations and reported that approximately half changed their recommendations after receiving RS results, with 33% recommending endocrine therapy alone instead of endocrine plus chemotherapy. However, only
medical oncologists who were already using the assay (16% response rate) were surveyed, thus biasing the results. Finally, no outcomes were reported providing no firm evidence of clinical utility.

**Patients with DCIS**

Ductal carcinoma in situ (DCIS) is breast cancer located in the lining of the milk ducts that has not yet invaded nearby tissues. It may progress to invasive cancer if untreated. The frequency of DCIS diagnosis in the U.S. has increased in tandem with the widespread use of screening mammography, accounting for about 20% of all newly diagnosed invasive plus noninvasive breast tumors. Recommended treatment is lumpectomy (mastectomy is also an option) with or without radiation treatment; post-surgical tamoxifen treatment is recommended for ER-positive DCIS, especially if excision alone is used. Because the overall rate of ipsilateral tumor recurrence (DCIS or invasive carcinoma) is about 25% at 10 years, it is believed many women are overtreated with radiation therapy. Thus, accurate prediction of recurrence risk may identify those women who may safely avoid radiation.

The Oncotype DX DCIS test uses information from 12 of the 21 genes assayed in the standard Oncotype DX test for early breast cancer to predict 10-year risk of local recurrence (DCIS or invasive carcinoma). The stated purpose is to help guide treatment decision-making in women with DCIS treated by local excision, with or without adjuvant tamoxifen therapy.

According to the Oncotype website, analyses from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14 study and the Habel et al. case-control study were used to select genes that predict the risk of recurrence independent of tamoxifen treatment and ER status. In a retrospective analysis of data and samples from patients in the prospective Eastern Cooperative Oncology Group E5194 study, the Oncotype DX Score for DCIS was compared with the 10-year recurrence risk in a subset of DCIS patients treated only with surgery and some with tamoxifen (n=327). DCIS Score was significantly associated with recurrence outcomes (HR: 2.31; 95% CI: 1.15, 4.59; p=0.02) whether or not patients were treated with tamoxifen. The standard Oncotype DX Score for early breast cancer was not associated with DCIS recurrence outcomes. These studies address the development of the Oncotype DX DCIS Score and the clinical validity (association of the test result with recurrence outcomes). Whether women are better categorized as to their recurrence risk by the Oncotype DX DCIS Score compared with standard clinical indicators of risk has not yet been addressed.

In another retrospective analysis, Rakovitch et al (2015) evaluated 571 tumor specimens with negative margins from a convenience cohort of patients with DCIS treated by breast-conserving surgery (lumpectomy) alone. Patients were drawn from a registry of 5752 women in Ontario, Canada, who were diagnosed with DCIS between 1994 and 2003. Median follow-up of the 571 women was 9.6 years. There were 100 local recurrence events (18% prevalence); 43 were DCIS (8% prevalence), and 57 were invasive cancer (10% prevalence). Oncotype DX® DCIS score was significantly associated with local recurrence outcomes (HR=2.15; 95% CI, 1.43 to 3.22). Sixty-two percent of patients were classified as low-risk, 17% as intermediate risk, and 21% as high risk. Corresponding 10-year local recurrence estimates were 13% (95% CI, 10 to 17), 33% (95% CI, 24 to 45), and 28% (95% CI, 20 to 38), respectively. Corresponding 10-year estimates for DCIS recurrence (5% [95% CI, 3 to 9]; 14% [95% CI, 8 to 24]; 14% [95% CI: 9 to 22], respectively) and for invasive
breast cancer recurrence (8% [95% CI, 6 to 12]; 21% [95% CI, 13 to 33]; 16% [95% CI, 9 to 25], respectively) were based on small numbers of events. It is unclear whether estimated recurrence risks for patients classified as low risk are low enough to forgo radiotherapy.

**MammaPrint**

MammaPrint, also called the 70-gene signature, is a prognostic test for women with ER-positive or ER-negative, lymph node-negative invasive breast cancer. The 2014 TEC Assessment reviewed available studies and found insufficient evidence to determine whether MammaPrint is better than conventional risk assessment tools in predicting recurrence. Limited technical-performance evaluation of the commercial version of the assay, using fresh frozen tumor samples suggested good reproducibility. In 2014, Sapino et al published a validation study of MammaPrint using FFPE tissue. In a validation set of 221 tumor samples, concordance of FFPE and frozen tissue low- and high-risk classification was 91.5% (95% CI, 86.9 to 94.5). Concordance of repeat analyses of the same tumor was 96%, and interlaboratory reproducibility (ie, between labs in the Netherlands and in California) was 96%.

**Studies of primarily node-negative disease**

In studies reviewed in the TEC Assessment, recurrence rates of patients classified by MammaPrint as low risk were 15% to 25%, likely too high for most patients and physicians to consider forgoing chemotherapy. Similarly, in 1 study, after Adjuvant! Online risk classification, patients reclassified as low risk by the 70-gene signature in either Adjuvant! Online risk group had 10-year DFS rates of 88% to 89%, with lower confidence limits of 74% to 77%. Patients reclassified as high risk had 10-year DFS rates of 69%, with lower confidence limits of 45% to 61% and upper confidence limits of 76% to 84%. Receiver operating characteristic (ROC) analyses suggested both small and large improvements in risk prediction with MammaPrint added to a conventional risk classifier (Adjuvant! Online).

Because initial studies had been conducted on samples from younger patients (age younger than 61 years), Wittner et al. studied a cohort of 100 lymph node-negative patients with a median age of 62.5 years and a median follow-up of 11.3 years. Twenty-seven low-risk patients by MammaPrint had distant metastasis-free survival at 10 years of 100%. However, the study was underpowered, and patients were heterogeneous in terms of ER-positivity (73%), endocrine therapy (25%), and chemotherapy (23%) making conclusions difficult. An additional small study of samples from women with lymph node-negative disease suggested that the 70-gene signature was an independent and significant predictor of distant metastases, but the small number of events limited conclusions.

Original validation studies included patients with both node-negative and node-positive disease. Mook et al. retrospectively evaluated 148 consecutive, node-negative, post-menopausal patients, with primarily ER-positive tumors; only 18% received 2 years of adjuvant tamoxifen and none chemotherapy. (45) For the 61% with good prognosis, 5-year distant metastasis-free survival (DMFS) probability was 93% (95% CI: 87-99%) whereas for those with poor prognosis DMFS was 72% (CI: 60-84%). The authors reported on concordance with Adjuvant! Online, but did not conduct a net reclassification analysis to determine additional impact of the MammaPrint signature on outcomes.
The Microarray Prognostics in Breast Cancer (RASTER) study was designed to assess feasibility of implementation and impact on treatment decisions of the MammaPrint 70-gene signature, as well as recurrence outcomes. Five-year follow-up results were presented at the 8th European Breast Cancer Conference. The study followed 427 node-negative, early-stage breast cancer patients who participated in the RASTER Study and had a 70-gene signature (MammaPrint), were published in 2013. Use of MammaPrint to help direct postsurgery treatment decisions were compared with Adjuvant! Online. Patients were aged 18 to 61 years and had a histologically-confirmed, unilateral, unifocal, primary operable, invasive adenocarcinoma of the breast. Median follow-up was 61.6 months. Eighty percent of patients were ER-positive. Discordant risk classifications occurred in 161 (38%) of 427 cases: 124 (29%) of 427 cases were discordant MammaPrint low risk and Adjuvant! Online high risk, and 37 (9%) of 427 cases were discordant MammaPrint high risk and Adjuvant! Online low risk. Use of MammaPrint reduced the proportion of Adjuvant! Online high-risk patients by 20% (87/427). Five-year distant recurrence-free interval (DRFI) probabilities were excellent for patients who were clinically high risk but had a low-risk score with MammaPrint, even in the absence of adjuvant systemic therapy. Patients originally classified high risk by Adjuvant! Online but low risk by the 70-gene signature (n=70) had a similarly high DDFS of 100%. The results of patients receiving adjuvant therapy are presented in Table 4. The results suggest that MammaPrint is a better prognostic classifier than standard clinical and pathological classifiers. However, the patient numbers are low, and event numbers very low, making firm conclusions difficult. Actual treatment decisions were based on restrictive Dutch guidelines from 2004 and patients’ and doctors’ preferences. Additionally, Adjuvant! Online risk estimates were calibrated for 10-year outcomes, whereas RASTER outcomes were at 5 years. Because most clinical relapses in lymph node-negative, ER-positive breast cancers do not occur until 5 or even 10 years after diagnosis, with or without the use of adjuvant therapy, study data should be considered not yet mature.

Table 4. Five-Year Results of the RASTER Study: DRFI in MammaPrint- and Adjuvant! Online-Classified Patients Who Received Adjuvant Systemic Treatment.

<table>
<thead>
<tr>
<th>70-gene signature category</th>
<th>Adjuvant Online! Category</th>
<th>AST</th>
<th>5-year DDFS (%. 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Low</td>
<td>AST</td>
<td>7/95 (7%) 95.3 (90.9 to100)</td>
</tr>
<tr>
<td>High</td>
<td>Low</td>
<td>32/37 (86%) 100.0 (100 to 100)</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>High</td>
<td>24/124 (44%) 98.4 (96.1 to 100)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>High</td>
<td>166/171 (97%) 89.8 (85.1 to 94.9)</td>
<td></td>
</tr>
</tbody>
</table>

AST: adjuvant systemic therapy, CI: confidence interval; DRFI distant recurrence-free interval.

Drukker et al (2014) reported additional comparisons between MammaPrint and clinical risk classifiers in RASTER patients. As measured by ROC analyses, MammaPrint improved prognostic performance of all 6 clinical classifiers studied (Adjuvant! Online, Nottingham Prognostic Index [NPI], St. Gallen [2003], Dutch National guidelines [2004 and 2012], and PREDICT Plus, a
clinicopathologic algorithm for estimating 5- and 10-year survival probabilities). However, area under the ROC curve for the best performing combination, MammaPrint and PREDICT Plus, was only 0.662. Five-year distant recurrence-free survival estimates in 158 untreated patients who were classified by MammaPrint as low risk are shown in Table 5. Among MammaPrint low-risk patients, 5-year survival estimates for patients classified as low or high risk by clinical risk classifiers were similar; only PREDICT Plus included the possibility of a less than 10% survival estimate for high-risk patients. Survival estimates for untreated patients classified as high risk by MammaPrint were not reported, limiting full comparison of these risk stratifiers.

Table 5. Five-Year Distant Recurrence-Free Survival Estimates of 158 Untreated RASTER Patients Classified as Low Risk by MammaPrint

<table>
<thead>
<tr>
<th>Low Risk</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjuvant! Online</td>
<td>5% 95.0</td>
<td>95% CI:</td>
<td>90.3 to 99.9</td>
<td>44% 100.0</td>
<td>100% CI:</td>
<td>100.0 to 100.0</td>
<td></td>
</tr>
<tr>
<td>Nottingham</td>
<td>9% 97.1</td>
<td>94.3 to 96.3</td>
<td>3% 100.0</td>
<td>100% CI:</td>
<td>100.0 to 100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>St. Gallen 2003</td>
<td>3% 98.2</td>
<td>94.8 to 96.3</td>
<td>63% 99.6</td>
<td>96.6% CI:</td>
<td>92.8 to 100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch National</td>
<td>9% 97.1</td>
<td>94.3 to 96.3</td>
<td>4% 100.0</td>
<td>100% CI:</td>
<td>100.0 to 100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PREDICT Plus</td>
<td>5% 98.8</td>
<td>96.3 to 99.6</td>
<td>47% 95.4</td>
<td>90.4% CI:</td>
<td>90.4 to 100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8% 97.6</td>
<td>94.9 to 94.1</td>
<td>11% 94.1</td>
<td>83.6% CI:</td>
<td>83.6 to 100.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval; % n: proportion of patients classified.

Studies of mixed or node-positive disease

In a study of node-positive disease, Mook et al. evaluated 241 patients with 1-3 positive nodes and primarily ER-positive, HER2-negative tumors treated variably. The 70-gene signature was a significant predictor of outcome. Reclassification analysis using Adjuvant! Online vs. MammaPrint showed significant additional discrimination of outcomes by the gene signature, but all were confounded by heterogeneous patient treatment. This study also updated the results of 106 patients with 1-3 positive nodes from the validation study, reporting 98% (95% CI: 94-100%) 10-year breast cancer-specific survival for good prognosis signatures vs. 64% (52-76%) for poor prognosis signatures; adjusted hazard ratio (HR): 3.63 (0.88–14.96, p=0.07. Based on these results, the ongoing MINDACT trial of MammaPrint was enlarged to include patients with 1-3 positive lymph nodes. Pilot phase results of the MINDACT trial were published in 2011 and showed successful implementation of the biomarker-stratified trial design and compliance with chemotherapy treatment according to MammaPrint risk of recurrence classification.

The 2012 I-SPY trial evaluated 237 patients with locally advanced disease (node-positive) by correlating imaging and MammaPrint signatures with outcomes of pathologic complete response (pCR) and recurrence-free survival (RFS). Despite having locally advanced disease, patients with 70-gene low-risk profiles tended not to respond to chemotherapy and to have good short-term RFS. Results are shown in Table 6.

Table 6. Results of I-SPY 1: MammaPrint 70-gene signature results and trial outcomes

<table>
<thead>
<tr>
<th>MammaPrint</th>
<th>Pathological complete response (pCR)</th>
<th>Recurrence-free survival (RFS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>
Breast Cancer- Assays of Genetic Expression in Tumor Tissue

Risk Category

<table>
<thead>
<tr>
<th>N (%)</th>
<th>Rate of pCR, % (n/N)</th>
<th>Odds ratio (p value)</th>
<th>3-yr RFS, % (n/N)</th>
<th>Hazard Ratio, pCR vs. no pCR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low 11 (9)</td>
<td>0% (0/11)</td>
<td>0.00</td>
<td>100% (11/11)</td>
<td>0.00 (-)</td>
</tr>
<tr>
<td>High 109 (91)</td>
<td>24% (25/105)</td>
<td>(0.02)</td>
<td>75% (80/105)</td>
<td>0.29* (0.07-0.82)</td>
</tr>
</tbody>
</table>

*denotes significant proportional hazard ratio (likelihood ratio p<0.05). A value of 0.00 indicates that there were no recurrences in this category among patients who had a pCR.

Other studies comprise primarily small case series and pooled re-analyses of subgroups from previously published retrospective studies. A pooled analysis of 964 patients from previously reported studies with pT1 tumors (<2 cm) included 84% with ER-positive tumors, 68% with HER2-negative tumors (no HER2 information on 23%), 27% with node-positive disease, 68% given no adjuvant treatment, and the rest treated variably. In these patients, overall distant metastasis-free survival at 10 years was 87% (95% CI: 84-91%) for good prognosis patients and 72% (66-78%) for poor prognosis patients. The hazard ratio was 2.7 (95% CI: 1.88–3.88, p<0.001). Results are confounded by nodal status, HER2 status, and adjuvant therapy.

Kunz conducted a pooled re-analysis of a subgroup of patients aged 35-55 years from previously published studies. Patients were 75% ER-positive, 45% node-positive; 60% were untreated and the rest treated variably. The 70-gene signature categorized 39% of patients as good prognosis; for these patients, the 10-year distant metastasis-free survival was 88% (95% CI: 84–92%). Bighin et al. reported difficulties in that nearly 25% of samples from 21 prospectively studied patients were not assessable by the 70-gene signature and that results lead to a change in clinical decision in fewer than 20% of cases.

Retel et al. reported a cost-effectiveness analysis that simulated the course of events in a hypothetical cohort of 1,000 patients aged 50 years with early, operable node-negative, ER-positive breast cancer, who are treated with 2.5 years of tamoxifen and 2.5 years of an aromatase inhibitor. The 70-gene signature was compared with Adjuvant! Online and St Gallen clinicopathologic classifiers. While all three strategies were clinically equally effective, St Gallen was more costly and the 70-gene signature was most cost-effective when quality-adjusted life-years were taken into account.

Saghatchian et al (2013) evaluated MammaPrint signatures of frozen tumor samples from patients who had 4 to 9 positive lymph nodes. Approximately half of patients were ER-positive, half were HER2-positive, and half had received adjuvant radiotherapy or chemotherapy. Seventy (40%) of 173 samples were classified as low risk by MammaPrint, and 103 (60%) were classified as high risk. With median follow-up of 8 years, 5-year breast cancer-specific survival in the low- and high-risk groups were 97% and 76%, respectively (log-rank test, p<0.01); 5-year distant metastasis-free survival was 87% and 63%, respectively (log-rank test, p=0.004). Survival estimates were reported without 95% CIs; it is therefore not possible to assess the degree of overlap between risk groups.

Ahn et al (2013) investigated the use of MammaPrint to further risk-stratify 82 ER-negative patients (56% lymph node–negative) who had Oncotype DX intermediate risk scores. Although MammaPrint...
risk classification was significantly associated with 10-year OS in multivariate analysis (log-rank test, p=0.013), this result was confounded by receipt of adjuvant chemotherapy, which also was significantly associated with OS (log-rank test, p=0.024).

To assess the impact of MammaPrint on treatment decision making, Cusumano et al (2014) distributed clinical information about 194 patients in European countries to multidisciplinary teams in 2 other countries (e.g., data from the Netherlands was sent to Belgium and Italy) first without and then with MammaPrint gene signatures. Eighty-six percent of patients were ER-positive, 88% were HER2-negative, and 66% were lymph node-negative. With the addition of MammaPrint signatures, treatment recommendations changed in 27% of patients, 22% from chemotherapy to no chemotherapy, and 35% from no chemotherapy to chemotherapy. In the subset of 453 ER-positive, HER2-negative patients, treatment advice changed in 32% of patients, with similar proportions changing from chemotherapy to no chemotherapy and vice versa. Other studies assessing the impacts of testing on treatment decision making also did not include survival or recurrence outcomes and are therefore considered uninformative for assessing clinical utility of MammaPrint.

Ongoing Trials

Section Summary

The majority of MammaPrint studies, including the early validation studies, suffered from confounding in heterogeneous sample populations. Subsequent pooled re-analyses of subpopulations controlled for one variable (e.g., nodal status), but confounding remained from other variables (e.g., treatment heterogeneity). Results for the 70-gene signature good prognosis patients have confidence intervals that extend into ranges that likely confer too much risk for patients and providers in the U.S. Because the test result is not a continuous numerical result, patients cannot view their result within the spectrum of good prognosis results and adjust their preferences accordingly. The recently presented RASTER (Microarray Prognostics in Breast Cancer) study represents an improved study design, and results suggest that MammaPrint may accurately reclassify early, node-negative breast cancer patients classified high risk for recurrence by clinical and pathologic variables to low risk, such that chemotherapy may not be necessary. However, the study is not yet published, patient numbers and events are too low for firm conclusions, and follow-up is not yet sufficiently mature.

Blueprint and TargetPrint

The BluePrint molecular subtyping profile was developed using 200 breast cancer specimens that had concordant ER, PR, and HER2 protein levels by IHC and TargetPrint mRNA readout. Using a 3-fold cross validation procedure, 80 genes thought to best discriminate the 3 molecular subtypes were identified. BluePrint was confirmed on 4 independent validation cohorts (total N=784), which included patients from a consecutive series of patients seen at the Netherlands Cancer Institute and treated with adjuvant tamoxifen monotherapy (n=274), a group of patients from the RASTER trial (n=100), and 2 publicly available data sets (n=410). Additionally, in 133 patients treated with neoadjuvant chemotherapy, the molecular subtyping profile was tested as a predictor of chemotherapy response. The authors concluded that use of BluePrint classification showed improved distribution of pCR among molecular subgroups compared with local pathology: 56% of
patients had a pCR in the basal-type subgroup, 3% in the MammaPrint low-risk, luminal-type subgroup, 11% in the MammaPrint high-risk, luminal-type subgroup, and 50% in the HER2-type subgroup. In a similar study, Whitworth et al (2014) reported reclassification of 94 (22%) of 426 patients with breast cancer who were classified by both IHC/fluorescence in situ hybridization (FISH) and BluePrint and treated with neoadjuvant chemotherapy. Six percent of BluePrint luminal-type patients achieved pCR compared with 10% of IHC/FISH hormone receptor–positive/HER2-negative patients; 53% of BluePrint HER2-positive patients achieved pCR compared with 38% of IHC/FISH HER2-positive patients (the majority of HER2-positive patients by either method received trastuzumab); and 35% of BluePrint basal-type patients achieved pCR compared with 37% of IHC/FISH “triple-negative” patients.

Nguyen et al (2012) undertook a comparison of molecular subtyping with BluePrint, MammaPrint, and TargetPrint to locally assess clinical subtyping using IHC and fluorescence FISH. The 3 gene expression assays were performed on fresh tumor tissue at Agendia Laboratories, blinded for pathologic and clinical data. IHC and FISH testing were performed according to local practice at 11 institutions in the U.S. and Europe. ER, PR, and HER2 assays were performed on 132 samples. Concordance between BluePrint and IHC and FISH testing was 94% for both basal-type and luminal-type subgroups, and 95% for HER2-type. Concordance between BluePrint and TargetPrint was 98% for the basal-type, 96% for the luminal-type, and 97% for the HER2-type subgroups.

Viale et al (2014) reported concordance between TargetPrint and IHC testing for ER, PR, and FISH for HER2 in the first 800 patients enrolled in the pilot phase of the MINDACT MammaPrint trial. For ER, positive and negative percent agreement between TargetPrint and central testing were 98% and 96%, respectively; positive (PPV) and negative predictive value (NPV) were 99% and 87%, respectively. For PR, positive and negative percent agreements were 83% and 91%, respectively; PPV and NPV were 97% and 59%, respectively. For HER2, positive and negative percent agreements were 75% and 99%, respectively; PPV and NPV were 91% and 97%, respectively.

Breast Cancer Index

The Breast Cancer Index is a simultaneous assessment of HOXB13:IL17BR (H/I) Index and the MGISM (Molecular Grade Index). The H/I ratio indicates estrogen-mediated signaling; MGI assesses tumor grade by measuring the expression of 5 cell-cycle genes and provides prognostic information in ER-positive patients regardless of nodal status. The 2014 TEC Assessment reviewed available studies for the original component assays. There was insufficient evidence to determine whether the H/I ratio is better than conventional risk assessment tools in predicting recurrence. Ten-year recurrence estimates of patients classified as low risk were 17% to 25%, likely too high for most patients and physicians to consider forgoing chemotherapy. Studies of the combination BCI are reviewed next. Ma et al. evaluated MGI along with H/I in 93 patients with lymph node–negative tumors who received adjuvant hormone therapy and found that each index modified the other’s predictive performance. High MGI was associated with significantly worse outcome only in patients with high H/I and vice versa. When the H/I Ratio and MGI were categorically combined into a single predictor, the estimates of 10-year distant metastasis-free survival were 98% (95% CI: 96-100%), 87% (77-99%), and 60% (47-78%) for the low, intermediate, and high-risk groups, respectively.
Jerevall et al. combined the H/I Ratio and MGI into a continuous risk model using 314 ER-positive, node-negative postmenopausal patients from the tamoxifen-only arm of an RCT. The continuous model was also categorized, resulting in proportions of low-, intermediate-, and high-risk patients similar to those reported in the Ma et al. study. This continuous predictor was tested in patients from the no adjuvant treatment arm (n=274) of the same clinical trial, with estimates of rates of distant metastasis at 10 years in the low-, intermediate-, and high-risk groups of 8.3% (95% CI: 4.7–14.4), 22.9% (14.5–35.2), and 28.5% (17.9–43.6), respectively. The estimates of breast cancer-specific death were 5.1% (95% CI: 1.3–8.7), 19.8% (10.0–28.6), and 28.8% (15.3–40.2). An independent population of otherwise similar but tamoxifen-treated patients was not tested.

Jankowitz et al. evaluated tumor samples from 265 ER-positive, lymph node (LN)-negative, tamoxifen-treated patients from a single academic institution’s cancer research registry. BCI categorized 55%, 21%, and 24% of patients as low, intermediate and high risk, respectively, for distant recurrence. The 10-year rates of distant recurrence were 6.6% (95% CI: 2.3-10.9%), 12.1% (95% CI: 2.7-21.5%), and 31.9% (95% CI: 19.9-43.9) and of breast cancer-specific mortality were 3.8%, 3.6% and 22.1% in low-, intermediate-, and high-risk groups, respectively. In a multivariate analysis, BCI was a significant predictor of distant recurrence and breast cancer-specific mortality. In a time-dependent (10-year) ROC curve analysis of recurrence risk, the addition of BCI to Adjuvant! Online risk prediction increased maximum predictive accuracy in all patients from 66% to 76% and in tamoxifen-only treated patients from 65% to 81%.

Sgroi et al (2013) examined 665 lymph node-negative, ER-positive, postmenopausal women receiving endocrine therapy but no chemotherapy in the ATAC trial. For patients in the low- and intermediate-risk groups, 10-year distant recurrence risks were 5% and approximately 19%, respectively, regardless of endocrine treatment (tamoxifen, anastrozole, or both). In the high-risk group, recurrence risk was lowest (22%) for patients taking anastrozole only –22%, comparable to the intermediate-risk group, and highest for patients taking tamoxifen only (37%), although these groups were small (54 and 55 patients, respectively).

Mammostrat Breast Cancer Test

Mammostrat is an immunohistochemistry (IHC) test intended to evaluate risk of breast cancer recurrence in postmenopausal, node-negative, ER-positive invasive breast cancer patients who will receive endocrine therapy and are considering adjuvant chemotherapy. The test employs 5 monoclonal antibodies to detect gene expression of proteins biologically independent of each other and not involved in cell proliferation, hormone receptor status, or growth/differentiation, thus potentially allowing integration with clinically routine biomarkers. A proprietary diagnostic algorithm is used to calculate a risk score and to classify patients into high-, moderate-, or low-risk categories.

One published study described the development of the assay but provides no information on technical performance (analytic validity). In a validation study in an independent cohort, a multivariable model predicted 50%, 70%, and 87% 5-year DFS for patients classified as high, moderate, and low prognostic risk, respectively, by the test results (p=0.0008). An additional study of the same trial samples used for Oncotype DX validation (NSABP B-14 and B-20 trials) found that among patients with early, node-negative breast cancer treated only with tamoxifen, those stratified by Mammostrat into low-, moderate-, and high-risk groups had recurrence-free survival.
estimates of 85%, 85%, and 73%, respectively. Both low- and high-risk groups benefited significantly from chemotherapy treatment, but high-risk patients benefited to a greater degree. The moderate-risk group was not well-separated from the low-risk group and thus, moderate-risk results do not appear to provide clinically useful information. A test for an interaction between chemotherapy and the risk group stratification was not significant (p=0.13).

Bartlett et al. used Mammostrat on 1,540 of 1,812 patient samples from a consecutive cohort for which minimum 9-year outcomes were available. The tested samples were from tamoxifen-treated patients; 568 of these were from node-negative patients treated only with tamoxifen and whose tumors were ER-positive. In the latter group, the distant recurrence rates at 10 years for low-, moderate-, and high-risk patients were 7.6% (95% CI: 4.6-10.5%), 16.3% (10.0-22.6%), and 20.9% (12.3-29.5%) respectively. In multivariable analysis, Mammostrat was not a significant predictor of recurrence-free survival in node-negative, ER-positive patients treated only with tamoxifen. However, when all patients (24% node-positive, 20% tumors >2.0 cm, 18% ER-negative, and 46% treated with chemotherapy) were included in a multivariable analysis, Mammostrat scores were independent predictors of recurrence-free survival (p=0.0007). In exploratory analyses of various subpopulations (e.g. node-negative vs. node-positive, ER-negative), Mammostrat appeared to perform similarly in terms of identifying risk groups. However, numbers of subsets were small.

In 2014, Stephen et al assessed the ability of Mammostrat and IHC4 to provide information on the risk of early (0-5 years) or late (5-10 years) distant recurrence. Tumor samples from 2 separate cohorts were analyzed: the Edinburgh Breast Conservation Series (n=1103) with median follow-up of 12.9 years, and the Tamoxifen Exemestane Adjuvant Multinational (TEAM) trial (n=3766) with median follow-up of 6.2 years. Patients had ER-positive disease and were treated with endocrine therapy without chemotherapy. Within the first 5 years after diagnosis, HRs comparing Mammostrat® high- with Mammostrat® low-risk patients were statistically significant only in the TEAM cohort, which had greater risk for relapse (greater mean tumor size, larger proportion of higher grade tumors, and greater mean number of positive lymph nodes) compared with the Edinburgh cohort. Measures of calibration (slope) and discrimination (R² statistic and index of discrimination) indicated that after 5 years (in the subset of patients who remained distant-recurrence free for at least 5 years, n=3920 [81%]), there was no evidence of an association between Mammostrat® scores and time to distant recurrence

**BreastOncPx**

The BreastOncPx test is a reverse transcriptase-polymerase chain reaction (RT-PCR) test performed on formalin-fixed, paraffin embedded tissue that measures the gene expression of 14 genes associated with key functions such as cell-cycle control, apoptosis, and DNA recombination and repair. The results are combined into a metastasis score, which is reported to be associated with the risk of distant metastases in patients who are node-negative and estrogen-receptor positive. Tutt et al. published information on the development and validation of the test; no information on analytic validity was provided. In order to develop a gene signature that was completely prognostic for distant recurrence and not confounded by treatment prediction, samples from untreated
patients with early breast cancer were used. The training set (n=142) was derived from a cohort diagnosed with lymph node-negative, stage T1 and T2 breast cancer from 1975 to 1986; ER-positive samples from patients who had had no systemic treatment were selected for analysis. Fourteen genes were eventually selected as most prognostic of time-to-distant metastasis and were given equal weighting in a summary metastasis score (MS). Using a single cutoff, patients are separated into high- and low-risk groups.

The 14-gene signature was validated on ER-positive samples (n=279) from a separate cohort of patients diagnosed with lymph node-negative primary breast cancer between 1975 and 2001. The estimated rates of distant metastasis-free survival were 72% (95% CI: 64-78%) for high-risk patients and 96% (95% CI: 90-99%) for low-risk patients at 10 years’ follow up. Overall 10-year survival for high- and low-risk patients was 68% (95 CI: 61% to 75%) and 91% (95% CI: 84 to 95%), respectively. After adjusting for age, tumor size, and tumor grade in a Cox multivariate analysis, the HRs for distant metastasis-free survival for the high- versus low-risk group were 4.02 (95% CI: 1.91-8.44) and 1.97 (95% CI: 1.28 to 3.04) for distant metastasis-free survival and overall survival, respectively. However, this difference in risk between groups was not maintained when the analysis was restricted to patients with tumors larger than 2 cm (p value for interaction 0.012).

ROC analysis of the continuous MS for distant metastasis and for death at 10 years, compared to Adjuvant! resulted in slightly higher area under the curves (AUCs) for the MS in each case: 0.715 vs. 0.661 for distant metastases, and 0.693 vs. 0.655 for death. MS was not added to Adjuvant! and compared to Adjuvant! alone.

NexCourse Breast IHC4

NexCourse Breast IHC4 evaluates the protein expression of ER/PR, HER2, and Ki-67 to provide a combined recurrence risk score. The assay technology uses quantitative image analysis to measure immunofluorescent signals, with results that can be combined in an algorithm to generate the recurrence risk score. The use of quantitative immunofluorescence is said to increase sensitivity, be more reproducible, and allow specific measurement of tumor cells.

Cuzick et al. evaluated 1,125 ER-positive patients from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial who did not receive adjuvant chemotherapy, already had the Oncotype DX Recurrence Score (RS) computed, and had adequate tissue for the IHC4 measurements. Of these, 793 were node-negative and 59 were HER2-positive (but were not treated with trastuzumab). A prognostic model that combined the 4 immunohistochemical markers was created (IHC4). In a model combining either IHC4 or Oncotype DX Rs with classical prognostic variables, the IHC4 score was found to be similar to the Oncotype DX RS, and little additional prognostic value was seen in the combined use of both scores. In a direct comparison the IHC4 score was modestly correlated with the Oncotype DX RS (r=0.72); the correlation was similar for node-negative patients (r=0.68). As an example, for a 1-2 cm, node-negative poorly differentiated tumor treated with anastrozole, 9-year distant recurrence at the 25th versus 75th percentiles for IHC4 and Oncotype DX were 7.6% versus 13.9% and 9.2% versus 13.4%, respectively. The IHC4 score was validated in a separate cohort of 786 ER-positive women, about half of whom received no endocrine treatment. The IHC4 score was significant for recurrence outcomes (HR: 4.1; 95% CI: 2.5-6.8).
Barton et al. assessed the clinical utility of IHC4 plus clinicopathologic factors (IHC4 + C) by comparison with Adjuvant! Online and the Nottingham Prognostic Index (NPI). The study prospectively gathered clinicopathologic data for consecutively treated postmenopausal patients (n=101 evaluable) with hormone receptor-positive, HER2-negative, LN-negative or -positive with 1-2 nodes, resected early breast cancer. Of 59 patients classified as intermediate-risk group by the NPI, IHC4 reclassified 24 to low risk and 13 to high risk. IHC4 reclassified 13 of 32 Adjuvant! high-risk patients to intermediate risk, and 3 of 32 to low risk. In addition, 15 of 26 Adjuvant! intermediate-risk patients were reclassified to low risk. No Adjuvant! low-risk patients were reclassified high risk.

In the Stephen study described above (see Mammostrat), HRs comparing the interquartile range of the continuous IHC4 score were statistically significant in both the Edinburgh and TEAM cohorts within the first 5 years after diagnosis. Measures of calibration and discrimination indicated that after 5 years, there was no evidence of an association between IHC4 scores and time to distant recurrence.

Prosigna /PAM50 Breast Cancer Intrinsic Subtype Classifier

The 2014 TEC Assessment reviewed development and validation studies of the PAM50 intrinsic subtype classifier and Prosigna; these studies are reviewed next. Only 2 studies of the marketed Prosigna test were identified, 1 of which reported analytic validity. A third study performed the commercial assay on 46 of the PAM50 genes, excluding 1 HER2-associated gene (GRB7) and 3 proliferation-associated genes (BIRCS [also called Survivin], MYBL2, CCNB1] that are given special weighting to generate the Prosigna recurrence of recurrence (ROR) score. These and other studies published after the 2014 TEC Assessment are reviewed next.

Initial development of the PAM50 breast cancer intrinsic classifier was reported in 2009 by Parker et al. The authors developed a qRT-PCR test based on a panel of 50 genes to identify the breast cancer “intrinsic” subtypes luminal A, luminal B, HER2-enriched, and basal-like, and to generate risk-of-relapse scores in node-negative patients who had not had systemic treatment for cancer. In an independent test set, the test using 3 categories of risk (low, intermediate, high) was significantly prognostic (log-rank test, p<0.001).

Nielsen et al (2010) compared the PAM50 classifier with standard clinicopathologic factors as represented by Adjuvant! Online and with models based on IHC for biomarkers of intrinsic subtypes. The study used samples from patients diagnosed between 1986 and 1992 with ER-positive, node-negative or node-positive breast cancer at higher risk (eg, with lymphovascular invasion), and treated with 5 years of tamoxifen but no adjuvant chemotherapy. In the node-negative population, Adjuvant! Online was inferior to all other biomarker models for predicting recurrence and disease-specific survival. A model including the PAM50 risk of recurrence (ROR) score that also incorporated the influence of proliferation and tumor size identified patients with a greater than 95% chance of remaining alive and disease-free beyond 10 years. A slightly different gene expression model best fit the node-positive population but did not identify a population at sufficiently low risk that adjuvant hormone therapy would likely be considered sufficient. Because the cohort used to generate the models evaluated in this study was biased toward higher risk early breast cancers, it is likely not generalizable. Nor did the authors clearly identify a final model for clinical use. Rather, the authors outlined potential additional studies.
Nielsen et al. (2014) assessed the analytical performance of Prosigna using the proprietary nCounter Analysis System (NanoString Technologies) at NanoString Technologies and 2 other laboratories. Each tumor sample had been classified by a pathologist as invasive carcinoma (of any type), and all sample testing was blinded. Assay precision was assessed by testing 5 tumor RNA samples 36 times at the 3 labs. SD across labs was less than 1 ROR unit on the 0 to 100 ROR scale. Reproducibility was measured by testing 43 FFPE tumor samples in the 3 labs. Measured total standard deviation including all sources of variation (ie, tissue processing and RNA processing variability) was 2.9 ROR units, indicating that Prosigna measures a difference of 6.8 points between continuous 2 ROR scores with 95% confidence. Concordance across the 3 labs for risk categorization in node-negative patients ranged from 88% (95% CI, 73 to 96) to 93% (95% CI, 80 to 98), and in node-positive patients, from 90% (95% CI, 77 to 96) to 95% (95% CI, 84 to 99).

In a study that supported FDA clearance of Prosigna, Gnant et al (2014) evaluated tumor samples from 1047 lymph node–negative patients who participated in the Austrian Breast and Colorectal Cancer Study Group’s trial 8 (ABCSG-8); this represented 28% of the original trial sample. ABCSG-8 randomized HR-positive, postmenopausal women with early stage breast cancer to 5 years of endocrine adjuvant therapy, either tamoxifen for 5 years or tamoxifen for 2 years followed by anastrozole for 3 years. Adjuvant or neoadjuvant chemotherapy was not allowed. In the Gnant et al (2014) study, both PAM50 subtype and Prosigna™ ROR class were associated with 10-year distant RFS, with CIs that overlapped slightly or not at all. Lower confidence limits for women in the luminal A and low-risk groups were around 94%, and upper confidence limits for luminal B and high-risk groups were approximately 90%. That is, the risk distinction seemed clinically useful. Filipits et al (2014) subsequently studied 919 patients who survived the first 5 years after treatment without recurrence. Fifteen-year late-distant DRS (ie, years 5-15) was 98%, 90%, and 86% in ROR low-, intermediate-, and high-risk groups, respectively.

Dowsett et al (2013) reported on groups from the ATAC trial stratified by subtype (luminal A or B) and by PAM50 ROR class, both with and without consideration of clinicopathologic factors. Among 739 lymph node–negative patients, 10-year distant RFS was 94% in 529 luminal A patients and 75% in 176 luminal B patients and was comparable with low- and high-risk ROR groups with or without clinical factors: 95%, 85%, and 70% in low-, intermediate-, and high-risk groups, respectively. An ROC analysis in 649 lymph node–negative, HER2-negative patients showed that PAM50 plus clinical factors had greater discriminatory ability than either risk predictor alone. In this study, the commercial assay was performed on 46 of the PAM50 genes (ROR46). The authors reported a correlation of 0.9989 between ROR50, which incorporated all PAM50 genes, and ROR46 risk classifications.

Two studies published in 2015 presented combined analyses of pretreatment FFPE tumor specimens from ABCSG-8 and ATAC trial monotherapy arms (TransATAC). Median follow-up was 10 years. Sestak et al examined the association between ROR score and late distant recurrence (5-10 years after diagnosis) in 2137 postmenopausal women (60% from ABCSG-8). Patients had hormone receptor–positive invasive breast cancer treated with only endocrine therapy (anastrozole or tamoxifen; no chemotherapy) for 5 years without recurrence. The majority of patients (74%) had node-negative disease (87% of patients with node-positive disease had 1 to 3 positive lymph nodes), and 92% were HER2-negative. ROR score was determined using a 46-gene subset of the PAM50 genes plus tumor size. Cut points differed from cut points used in the FDA-approved
version of the test, designed to assess recurrence risk in the first 10 years after diagnosis (years 0-10). In this study, ROR score less than 26 identified patients with low risk of distant recurrence (<10% risk); ROR score 26 to 68 identified patients with intermediate risk (10%-20% risk); and ROR score greater than 68 identified patients with high risk (>20% risk) in both node-negative and node-positive patients. Fifty-five percent of women were categorized as low risk, 25% as intermediate risk, and 20% as high risk. Kaplan-Meier estimated risks for late distant recurrence in node-negative patients were 2.3% (95% CI, 1.3 to 3.5), 8.5% (95% CI, 5.9 to 12.1), and 9.3% (95% CI, 5.5 to 15.5), respectively. In node-positive patients, estimated risks were 3.3% (95% CI, 1.2 to 8.6), 7.8% (95% CI, 4.4 to 13.8), and 20.9% (95% CI, 16.1 to 26.9) in low-, intermediate-, and high-risk groups, respectively.

Gnant et al (2015) evaluated FFPE tissue specimens from 543 patients in the ABCSG-8 and ATAC trials who had 1 to 3 positive lymph nodes.103 The primary end point was distant recurrence-free survival, defined as the interval from randomization until distant recurrence or death due to breast cancer. Investigators developed a Clinical Treatment Score (CTS) that integrated nodal status, tumor size, histopathologic grade, patient age, and type of endocrine therapy received (anastrozole or tamoxifen) into a summary score. Risk classification by CTS was compared with and without ROR in subsets of patients with 1 positive lymph node (n=331) and with 2 to 3 positive lymph nodes (n=212). ROR cut points for defining risk groups differed from cut points used in the FDA-approved version of the test, which were defined by Gnant et al (2014), discussed above. Among patients with 1 positive node, 40% were categorized as low risk, 32% as intermediate risk, and 28% as high risk. Kaplan-Meier estimates for 10-year distant recurrence or death from breast cancer were 6.6% (95% CI, 3.3 to 12.8), 15.6% (95% CI, 9.5 to 25.0), and 25.6% (95% CI, 17.5 to 36.0), respectively. Because the upper bound of the 95% CI for patients categorized as low risk exceeded 10%, usefulness of these risk distinctions is uncertain. For patients with 2 to 3 positive nodes, low and intermediate risk groups were combined due to small numbers of patients and events in the low-risk group; 39% of patients were categorized as low/intermediate risk, and 61% as high risk. Ten-year distant RFS estimates were 12.6% (95% CI, 6.6 to 22.8) and 33.7% (95% CI, 25.5 to 43.8), respectively. When ROR, either as a continuous or a categorical variable, was added to CTS, prognostic information was improved (changes in likelihood ratios were statistically significant) compared with CTS alone for all nodal subgroups, including node-negative patients. Liu et al (2015) assessed the prognostic and predictive value of PAM50–determined intrinsic subtypes and ROR scores in 1094 breast tumor samples from the National Cancer Institute of Canada’s MA.21 trial. MA.21 was an international, phase 3 trial that compared taxane and nontaxane chemotherapy in 2104 premenopausal or postmenopausal women 60 years of age or younger with node-positive or high-risk node-negative breast cancer. Patients were stratified by type of surgery (partial or total mastectomy), number of positive axillary lymph nodes, and ER status. Approximately 60% of patients were ER-positive, and approximately 60% received adjuvant endocrine therapy. PAM50 subtypes and ROR scores were determined using the nCounter Analysis system. Of all samples tested (52% of patients randomized), 3%, 18%, and 79% were classified as ROR low-, intermediate-, and high-risk, respectively. In multivariate analysis, ROR score on a continuous scale was statistically associated with RFS, but categorical ROR was associated with neither RFS nor survival by treatment group (ie, neither prognostic nor predictive). Intrinsic
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subtypes were associated with RFS but were not predictive of treatment outcomes. The authors stated:

“The characteristics of the study population of MA.21, which includes more high-risk breast cancer patients, are different from those used for the development and validation of the NanoString PAM50 ROR score classification. Thus, we suggest that researchers need to be cautious when applying the ROR risk classification in different study populations. Compared with ROR score, intrinsic subtype is expected to be more reliable for predicting clinical outcome and response to therapies in different breast cancer populations as it is based on the fundamental biology of breast cancer, whereas the ROR algorithm was optimized against outcome in a specific population.”

Martin et al (2015) evaluated the impact of ROR on treatment decision making in patients with ER-positive, HER2-negative, node-negative breast cancer. Because survival or recurrence outcomes were not reported, the study is considered uninformative for assessing clinical utility of Prosigna.

BreastPRS

BreastPRS is a gene expression assay that analyzes 200 genes and was validated in a meta-analysis of publically available genomic datasets. BreastPRS is a binary assay which stratifies patients into low- and high-risk groups.

D’Alfonso et al (2013) sought to translate a previously published validation study of BreastPRS, using fresh-frozen tissue, to FFPE tumor samples. The authors compared BreastPRS to Oncotype DX and correlated recurrence scores with clinicopathologic features. A linear relationship of BreastPRS prognostic scores between fresh-frozen and FFPE formats was observed. Using publically available whole genome profiles from a series of untreated ER-positive, node negative patients, investigators assessed the ability of BreastPRS to reclassify Oncotype DX intermediate-risk patients into high- or low-risk categories with clinically significant differences in outcomes. BreastPRS prognosis scores were compared with Oncotype DX recurrence scores in 246 patients with invasive breast carcinoma and known Oncotype DX results. Using this series, a 120-gene Oncotype DX approximation algorithm to predict Oncotype DX risk groups was then applied to a series of untreated, ER-positive, node-negative patients from previously published studies with known clinical outcomes. Of 30 high-risk Oncotype DX cases, 27 (90%) were classified as high-risk by BreastPRS, and 95 low-risk Oncotype DX cases (76%) were classified as low-risk by BreastPRS. The correlation of recurrence score and risk group between Oncotype DX and BreastPRS was statistically significant (p<0.001). Fifty-nine (23%) of 260 patients from 4 previously published studies were classified as intermediate-risk when the 120-gene Oncotype DX approximation algorithm was applied.

BreastPRS reclassified the 59 patients into binary risk groups (high vs low risk), with 23 (39%) patients classified as low risk and 36 (61%) as high risk (HR for a high-risk classification, 3.64; 95% CI, 1.40 to 9.50; p=0.029). Ten-year RFS was 90% in the low-risk group and 60% in the high-risk group. The authors concluded that BreastPRS prognosis score is comparable with Oncotype DX recurrence score and can reclassify Oncotype DX intermediate-risk patients into 2 groups with clinically significant differences in RFS.

EndoPredict

Poremba et al (2014) assessed the impact of tissue handling on EndoPredict (EP) test results. In analysis of 138 EndoPredict assays, time to fixation up to 12 hours, fixation time up to 5 days,
tumor cell content from 15% to 95%, and section storage time up to 12 months at 4°C or 20°C did not negatively impact results. This conclusion was based on correlations with test results from paired tissue samples with different storage conditions or different tumor cell content. It is unclear whether tests were conducted at more than 1 laboratory.

Bertucci et al (2014) evaluated 553 ER+/HER2-negative breast cancers treated with anthracycline-based neoadjuvant chemotherapy. Fifty-one percent of samples were classified as EndoPredict® low-risk with a pCR rate of 7%; 49% of samples were classified as EndoPredict® high-risk with a pCR rate of 17%. Estimated 5-year disease-free survival was 88% (95% CI, 81 to 95) in the EndoPredict® low-risk group and 73% (95% CI, 63 to 85) in the EndoPredict® high-risk group.

Varga et al (2013) analyzed the EndoPredict (EP) test in 34 hormone positive, invasive breast cancer cases and compared the EndoPredict (EP) scores with the Oncotype DX recurrence scores (RS) obtained from the same cancer samples. EP classified 11 patients as low risk and 23 patients as high risk, whereas RS classified 15 patients as low risk, 10 patients as intermediate risk, and 9 patients as high risk. There were major discrepancies in 6 of 34 cases (18%), with low-risk RS classified as high risk by EP in 6 cases. When the RS intermediate- and high-risk groups were combined, the concordance between both tests was 76%. The clinical relevance of these discrepant test results with respect to outcome is unknown.

Martin et al (2013) assessed tumor samples from 566 ER-positive, HER2-negative patients who participated in the GEICAM 9906 RCT. GEICAM 9906 compared 2 adjuvant chemotherapy regimens in 1246 women who had lymph node-positive disease: six 21-day cycles of 5-fluorouracil, epirubicin, and cyclophosphamide (FEC) or four 21-day cycles of FEC followed by 8 weekly courses of paclitaxel (FEC-P). EPclin score was calculated by combining EP score (RT-PCR assay of 8 genes) with nodal status and tumor size. EP was successfully assayed in 555 (98%) of 566 tumor samples. Twenty-five percent (n=141) of samples were classified as low risk by EP score, and 75% (n=414) were high risk; 10-year metastasis-free survival was 93% in the low-risk group and 70% in the high-risk group (HR for metastasis or death in the high- vs low-risk group, 4.8; 95% CI, 2.5 to 9.6; log-rank test, p<0.001). Thirteen percent (n=74) of samples were classified as low risk by EPclin score, and 87% (n=481) were classified as high risk; 10-year metastasis-free survival was 100% in the low-risk group and 72% in the high-risk group.

Dubsky et al (2013) examined predictive ability of EP and EPclin for early (0-5 years) and late (>5 years postdiagnosis) disease recurrence. Tumor samples from chemotherapy-untreated, ER-positive, HER2-negative patients who participated in 1 of 2 RCTs (ABCSG6 or ABCSG8) were assayed (total N=1702). In the trials, patients received either tamoxifen for 5 years or tamoxifen for 2 years followed by anastrozole for 3 years. Forty-nine percent (n=832) of patients were classified as low risk by EP score, and 51% (n=870) were classified as high risk. Only relative estimates (ie, HRs) of distant recurrence were reported. In comparison with low-risk patients, high-risk patients had an almost 3-fold increase in the risk of recurrence in the first 5 years after diagnosis (HR=2.80; 95% CI, 1.81 to 4.34; log-rank test, p<0.001) and a slightly increased risk after 5 years (in those who survived 5 years; HR=3.28; 95% CI, 1.48 to 7.24; log-rank test, p=0.002). By EPclin, 1066 (63%) of 1702 patients were classified as low risk, and 636 (37%) were classified as high risk. In comparison with low-risk patients, high-risk patients had an almost 5-fold risk of recurrence within the first 5 years (HR=4.82; 95% CI, 3.12 to 7.44; log-rank test, p<0.001) and a more than 6-fold increased risk...
of recurrence after 5 years (HR=6.26; 95% CI, 2.72 to 14.36; log-rank test, p<0.001). Given the discrepancy in risk classification between EP and EPclin, and the incomplete reporting of recurrence and survival outcomes by risk groups, this evidence is insufficient to demonstrate improved prognostic accuracy for individual patients with EndoPredict.

Test Comparison Studies

Sgroi et al (2013) compared the Breast Cancer Index and Oncotype DX in 665 lymph node–negative women receiving endocrine therapy but not chemotherapy in the ATAC trial. The distribution of patients across risk groups was similar. For patients receiving tamoxifen alone or in combination with anastrozole, 10-year distant recurrence risk estimates by the 2 tests were similar within risk groups. In the anastrozole group, the Breast Cancer IndexSM was a better predictor of risk: 5% of Breast Cancer IndexSM low-risk patients had distant recurrence compared with 9% of Oncotype DX low-risk patients, and 22% of Breast Cancer IndexSM high-risk patients had distant recurrence compared with 13% of Oncotype DX high-risk patients. Importantly, these values were reported without 95% CIs; it is therefore not possible to assess the degree of overlap between risk groups.

Dowsett et al (2013) compared PAM50 ROR score to the Oncotype Dx 21-gene RS, Breast IHC4, and a clinical treatment score (CTS). Patients had ER-positive, primary breast disease treated with anastrozole or tamoxifen in the ATAC trial (a double-blinded, Phase 3 clinical trial that was designed to compare the ability of anastrozole, tamoxifen, and the 2 drugs in combination to prevent breast cancer recurrence in postmenopausal women with HR-positive tumors). Lymph node-negative and positive patients were included. mRNA from 1017 patients was assessed for ROR, and likelihood ratio tests and concordance indices were used to assess the prognostic information provided beyond that of a CTS, RS, ROR, or IHC4. The CTS integrated prognostic information from nodal status, tumor size, histopathologic grade, age and anastrozole or tamoxifen treatment. The authors concluded that the ROR added significant prognostic information beyond CTS in all patients (p<0.001), and in all 4 subgroups: lymph node negative, lymph node positive, HER2 negative, and HER2 negative/node-negative, and that more information was added by ROR than RS. More patients scored as high risk of recurrence and fewer as intermediate risk by ROR than RS. Prognostic information provided by ROR score and IHC4 was similar.

Hornberger et al (2012) conducted a systematic review on the clinical validity, clinical utility, change in clinical practice, and economic implications of early-stage breast cancer stratifiers. Fifty-six articles published original evidence addressing the 21-gene recurrence score (Oncotype DX) (n=31), 70-gene signature (MammaPrint) (n=14), Adjuvant! Online (n=12), 5-antibody immunohistochemistry panel (Mammostrat) (n=3), and 14-gene signature (BreastOncPx) (n =1). Oncotype Dx recurrence score satisfied level 1 evidence for estimating distant recurrence risk (DRR), OS, and response to adjuvant chemotherapy, and level 2 evidence for estimating local recurrence risk. Mammastrat and MammaPrint satisfied level 2 evidence for estimating DRR and OS. Adjuvant! Online satisfied level 2 evidence for estimating DRR, OS, and chemotherapy response. BreastOncPx satisfied level 3 evidence for predicting DRR and OS. Ten studies reported changes in clinical practice patterns using Oncotype DX; overall, Oncotype DX was associated with change in treatment recommendations and/or decisions in 21% to 74% of cases.
Fan et al. used 5 gene expression classifiers to evaluate a single set of samples from 295 women with stage I or II breast cancer, variable node involvement, and variable endocrine or chemotherapy treatment. The classifiers included the 21-gene Recurrence Score, the 70-gene signature, the H/I ratio, and the intrinsic subtype classifier (similar to the commercially available PAM50). Most highly correlated were the 21-gene Recurrence Score and the 70-gene signature at a Cramer’s V of 0.6 (scale 0 to 1 with 1 indicating perfect agreement). More specifically, 81 of the 103 samples with a Recurrence Score of low or intermediate risk were classified as having a low risk 70-gene profile. Restricting the analysis to the 225 ER-positive samples slightly reduced the correlation. The analysis was not further restricted to node-negative patients, the present indication for both tests.

Espinosa et al. compared the 21-gene Recurrence Score (Oncotype DX), the 70-gene signature (MammaPrint), and the 2-gene ratio (H/I Ratio) in 153 patients with ER-positive breast cancer treated with adjuvant tamoxifen. Thirty-eight percent of these patients were node-positive, and 63% were additionally treated with chemotherapy. Distant metastasis-free survival for the Recurrence Score profile was 98% for low-risk patients versus 81% intermediate risk versus 69% high-risk; for the 70-gene signature the estimates were 95% good prognosis versus 66% poor prognosis; and for the 2-gene ratio, 86% favorable versus 70% unfavorable. There was a good correlation between the 21-gene Recurrence Score and the 70-gene signature (Cramer’s V=0.6). Slightly more variation in distant metastasis-free survival was explained by the combination of the 21-gene Recurrence Score and either Adjuvant! Online (25.8+1.4) or the Nottingham Prognostic Index (NPI; 23.7+1.5) than by the combination of the 70-gene signature with Adjuvant! Online (23.1+1.2) or the NPI (22.4+1.3), but the differences were very small and any combination was significantly better than any test or clinicopathologic classifier alone.

Two recent papers compared the Oncotype DX and other gene expression profiles. Kelly et al. evaluated Oncotype DX and PAM50 in 108 cases and found good agreement between the 2 assays for high- and low-prognostic risk assignment, but PAM50 assigned about half of Oncotype DX intermediate-risk patients to the PAM50 luminal A (low risk) category. Prat et al. evaluated several gene expression tests of interest including Oncotype DX, PAM50 and MammaPrint in 594 cases and found all predictors were significantly correlated (Pearson correlation range: 0.36-0.79; p<0.0001 for each comparison).

Additional Applications

Based on a study published in May 2008 that compared the Oncotype DX ER and PR results to traditional IHC results, Genomic Health is now including the quantitative ER and PR component results in the Oncotype DX 21-gene profile report. The study reported 90% or better concordance between the 2 assays but that quantitative ER by Oncotype DX was more strongly associated with disease recurrence than the IHC results. However, ER and PR analysis is traditionally conducted during pathology examination of all breast cancer biopsies, whereas Oncotype DX is indicated only for known ER-positive tumors, after the pathology examination is complete, the patient meets specific criteria, and patient and physician are considering preferences for risk and chemotherapy. Thus, Oncotype DX should not be ordered as a substitute for ER and PR IHC. Additionally, accepted guidelines for ER and PR testing outline standards for high-quality IHC testing and do not recommend confirmatory testing; thus the 21-gene RS should not be ordered to confirm ER/PR IHC
results. A subsequent study by Khoury et al (2015) reported better correlation (for overall data) between IHC and Oncotype DX® for PR (Spearman correlation, 0.91) than for ER (Spearman correlation, 0.65), but worse concordance (at various cut points) for PR than for ER (99% vs 88%, respectively).

Similarly, guidelines for HER2 testing specify IHC and/or fluorescence in situ hybridization (FISH) methods. The HER2 component of the 21-gene assay has been shown in one large study to strongly correlate with FISH results, but significant discrepancies have been noted in another. As a result, and without evaluation and support from guidelines, it has been recommended that the 21-gene assay not be ordered to determine or confirm HER2.

No published literature on the use of gene expression profiling in men with breast cancer was identified.

Investigators have examined the ability of gene expression tests to provide risk information for locoregional recurrence. Drukker et al (2014) applied MammaPrint to 1053 tumor specimens from 1848 patients enrolled in 8 previous MammaPrint studies. The majority of patients had ER-positive, HER2-negative disease; approximately half of patients had positive axillary lymph nodes. The majority of patients received radiotherapy and did not receive adjuvant chemotherapy; approximately half received adjuvant endocrine therapy. At median follow-up of 9 years, estimated 10-year locoregional recurrence risk was 13% (95% CI, 10 to 16) for 492 patients categorized as MammaPrint high-risk versus 6% (95% CI, 4 to 9) for 561 MammaPrint® low-risk patients. This association was observed during the first 5 years after diagnosis, but not during years 5 to 10. Recurrence stratified by MammaPrint risk class was not associated with primary locoregional treatment (ie, not predictive of treatment response).

Fitzal et al (2015) evaluated local recurrence using EndoPredict in breast tumor samples from 1324 patients who had participated in the ABCSG-8 trial (29% of enrolled patients), which compared adjuvant endocrine therapy regimens. The majority of patients had node-negative, ER-positive disease and received breast-conserving surgery and radiotherapy; approximately half of patients received adjuvant endocrine therapy. At median follow-up of 6 years, Kaplan-Meier estimated 10-year risk of local RFS was 96% (91% reported in the article abstract) among 683 patients classified by EndoPredict as high risk versus 99% among 641 patients classified by EndoPredict® as low risk. EndoPredict risk groups were not associated with treatment outcomes.

Some currently unpublished trials that might influence this policy are listed in Table 7.

Table 1. Summary of Key Trials

<table>
<thead>
<tr>
<th>NCT No.</th>
<th>Trial Name</th>
<th>Planned Enrollment</th>
<th>Completion Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NCT00433589</td>
<td>MINDACT (Microarray In Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy): A Prospective, Randomized Study Comparing the 70-Gene Signature With the</td>
<td>6600</td>
<td>Mar 2015</td>
</tr>
</tbody>
</table>
Common Clinical-Pathological Criteria in Selecting Patients for Adjuvant Chemotherapy in Breast Cancer With 0 to 3 Positive Nodes

<table>
<thead>
<tr>
<th>Trial ID</th>
<th>Title</th>
<th>Participants</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00310180</td>
<td>Program for the Assessment of Clinical Cancer Tests (PACCT-1): Trial Assigning Individualized Options for Treatment: The TAILORx Trial</td>
<td>11,248</td>
<td>Dec 2017</td>
</tr>
<tr>
<td>NCT01272037</td>
<td>A Phase III, Randomized Clinical Trial of Standard Adjuvant Endocrine Therapy +/- Chemotherapy in Patients With 1-3 Positive Nodes, Hormone Receptor-Positive and HER2-Negative Breast Cancer With Recurrence Score (RS) of 25 or Less. RxPONDER: A Clinical Trial Rx for Positive Node, Endocrine Responsive Breast Cancer</td>
<td>5000</td>
<td>Feb 2022</td>
</tr>
<tr>
<td>NCT02400190</td>
<td>The IDEA Study (Individualized Decisions for Endocrine Therapy Alone)</td>
<td>200</td>
<td>Mar 2026</td>
</tr>
</tbody>
</table>

NCT: national clinical trial.
* Final data collection date for primary outcome measure.

Clinical Input Received through Physician Specialty Societies and Academic Medical Centers

In response to requests, input was received from 1 physician specialty society and 4 academic medical centers while this policy was under review in 2008. While the various physician specialty societies and academic medical centers may collaborate with and make recommendations during this process, through the provision of appropriate reviewers, input received does not represent an endorsement or position statement by the physician specialty societies or academic medical centers, unless otherwise noted. A clear majority of the reviewers agreed with the policy conclusions.

Practice Guidelines and Position Statements

National Comprehensive Cancer Network

Current National Comprehensive Cancer Network (NCCN) guidelines for breast cancer (version v.2.2015) do not include any gene expression signature tests. However, pending results of prospective RCTs for Oncotype DX (TAILORx) and MammaPrint (MINDACT), Oncotype DX may be considered an option when evaluating patients who have invasive breast cancer with all of the following features (category 2A recommendation):

- Hormone receptor-positive;
- HER2-negative;
- Node-negative OR not greater than 2 mm axillary node metastasis; AND
- Size of 0.6–1 cm with unfavorable features OR larger than 1 cm.

For patients with node-positive, hormone receptor–positive, HER2-negative disease, NCCN guidelines include the following footnote:
"The 21 gene RT-PCR [reverse transcriptase–polymerase chain reaction] assay recurrence score can be considered in select patients with 1-3 involved ipsilateral axillary lymph nodes to guide the addition of combination chemotherapy to standard hormone therapy. A retrospective analysis of a prospective randomized trial suggests that the test is predictive in this group similar to its performance in node-negative disease."

The NCCN Guideline Panel emphasized that Oncotype DX recurrence score should be used for decision making only in the context of other elements of risk stratification for an individual patient.

American Society of Clinical Oncology

The 2007 American Society of Clinical Oncology (ASCO) guidelines indicate that “In newly diagnosed patients with node-negative, estrogen-receptor positive breast cancer, the Oncotype DX assay can be used to predict the risk of recurrence in patients treated with tamoxifen.”

St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer. The 2015 St. Gallen expert panel focused on “providing a practical approach to the allocation of available therapies” based on tumor factors, such as hormone receptors, HER2 status, and metastatic potential as reflected in measures of proliferation and anatomic extent of disease, and patient factors, such as menopausal status, age, comorbidity and patient preference.

- For prognosis, the Panel considered the role of multiparameter molecular marker assays separately in years 1 to 5 and beyond 5 years. “Oncotype DX®, MammaPrint®, PAM-50 ROR® score, EndoPredict, and the Breast Cancer Index® were all considered usefully prognostic for years 1-5. Beyond 5 years, the Panel was divided almost equally on the prognostic value of Oncotype DX, EndoPredict, and the Breast Cancer Index®. PAM50 ROR® score was agreed to be clearly prognostic beyond 5 years, and a clear majority rejected the prognostic value of MammaPrint in this time period.
- Only Oncotype DX commanded a majority in favor of its value in predicting the usefulness of chemotherapy.”

The Panel noted that threshold values for decision making about cytotoxic chemotherapy in patients with luminal disease had not been established for any of the tests. “Multi-parameter molecular assays are expensive and therefore unavailable in much of the world.”

U.S. Preventive Services Task Force

Not applicable

Gene expression testing and other prognostic tests (eg, immunohistochemistry) of breast cancer tumor tissue are not preventive services.

Medicare National Coverage

There is no national coverage determination (NCD). In the absence of an NCD, coverage decisions are left to the discretion of local Medicare carriers.
In November 2014, Palmetto GBA issued a local coverage determination for the Breast Cancer Index. Effective November 3, 2014, the policy limits coverage of the Breast Cancer Index\textsuperscript{SM} to patients who meet the following criteria:

- Post-menopausal female with non-relapsed, ER+ breast cancer; AND
- Is completing 5 years of tamoxifen therapy; AND
- Patient must be eligible for consideration of extended endocrine therapy based on published clinical trial data or practice guidelines; AND
- Physician or patient is concerned about continuing anti-hormonal therapy because of documented meaningful toxicity or possible significant patient-specific side effects; AND
- The test results will be discussed with the patient (including the limitations of the testing method, the risks and benefits of either continuing or stopping the therapy based on the test, and current cancer management guidelines).

VII. Important Reminder

The purpose of this Medical Policy is to provide a guide to coverage. This Medical Policy is not intended to dictate to providers how to practice medicine. Nothing in this Medical Policy is intended to discourage or prohibit providing other medical advice or treatment deemed appropriate by the treating physician.

Benefit determinations are subject to applicable member contract language. To the extent there are any conflicts between these guidelines and the contract language, the contract language will control.

This Medical Policy has been developed through consideration of the medical necessity criteria under Hawaii’s Patients’ Bill of Rights and Responsibilities Act (Hawaii Revised Statutes §432E-1.4), generally accepted standards of medical practice and review of medical literature and government approval status. HMSA has determined that services not covered under this Medical Policy will not be medically necessary under Hawaii law in most cases. If a treating physician disagrees with HMSA’s determination as to medical necessity in a given case, the physician may request that HMSA reconsider the application of the medical necessity criteria to the case at issue in light of any supporting documentation.

VIII. References

2. Blue Cross and Blue Shield Association Technology Evaluation Center (TEC). Gene expression profiling in women with lymph node negative breast cancer to select adjuvant chemotherapy. TEC Assessments 2014; Volume 29; (Tab TBD).
Breast Cancer- Assays of Genetic Expression in Tumor Tissue

14. Dowsett M. on Behalf of the ATAC Trialists Group. Analysis of time to recurrence in the ATAC (arimidex, tamoxifen, alone or in combination) trial according to estrogen receptor and progesterone receptor status. Twenty-sixth Annual San Antonio Breast Cancer Symposium 2003


Breast Cancer - Assays of Genetic Expression in Tumor Tissue


77. Viale G, Slaets L, Bogaerts J et al. High concordance of protein (by IHC), gene (by FISH; HER2 only), and microarray readout (by TargetPrint) of ER, PgR, and HER2: results from the EORTC 10041/BIG 03-04 MINDACT trial. Ann Oncol 2014; 25(4):816-23.


Appendix.


<table>
<thead>
<tr>
<th>Result</th>
<th>Immunohistochemistry</th>
<th>Fluorescence In Situ Hybridization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0 or 1+: No staining or faint/barely perceptible, incomplete membrane staining in any proportion of tumor cells</td>
<td>Ratio of $\text{HER2} / \text{CEP17}^a &lt; 2.0$ AND Average $\text{HER2}$ copy number &lt; 4.0 signals per cell OR Average $\text{HER2}$ copy number &lt; 4.0 signals per cell$^b$</td>
</tr>
<tr>
<td>Positive</td>
<td>3+: At least 10% of tumor cells exhibit complete, intense, circumferential membrane staining</td>
<td>Ratio of $\text{HER2} / \text{CEP17} &gt; 2.0$ OR Ratio of $\text{HER2} / \text{CEP17}$ is &lt; 2.0 AND Average $\text{HER2}$ copy number ≥ 6.0 signals per cell OR Average $\text{HER2}$ copy number ≥ 6.0 signals per cell$^b$</td>
</tr>
<tr>
<td>Equivocal</td>
<td>2+: Circumferential membrane staining that is either: • incomplete and/or weak/moderate within &gt; 10% of tumor cells, or • complete and intense within ≤10% of tumor cells</td>
<td>Ratio of $\text{HER2} / \text{CEP 17} &lt; 2.0$ AND Average $\text{HER2}$ copy number ≥ 4.0 and &lt; 6.0 signals per cell OR Average $\text{HER2}$ copy number ≥ 4.0 and &lt; 6.0 signals per cell$^b$</td>
</tr>
</tbody>
</table>

ASCO: American Society of Clinical Oncology; CAP: College of American Pathologists; CEP: chromosome enumeration probe; HER2: human epidermal growth factor receptor 2.

$^a$ CEP 17 is a centromeric probe for chromosome 17 (internal control probe).

$^b$ Signals per cell for test systems without an internal central probe.