IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

An important part of treatment planning for women with early stage breast cancer involves determining the potential benefit from adjuvant cytotoxic chemotherapy, using predictive information. Tests of genetic expression in tumor tissue have been proposed as techniques to determine prognosis (risk of recurrence) thereby providing additional information to guide treatment decisions for patients with breast cancer.

Background

For women with early stage breast cancer, adjuvant chemotherapy provides the same proportional benefit regardless of prognosis. However, the absolute benefit of chemotherapy depends on the baseline risk for recurrence. For example, women with the best prognosis have small tumors, are estrogen receptor-positive (ER+), and lymph node negative (N-). 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
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.

Several panels of gene expression markers (‘signatures’) have been identified that appear to predict the baseline risk of breast cancer recurrence after surgery, radiation therapy, and hormonal therapy (for hormone receptor-positive tumors) in women with node-negative disease. The available gene expression tests include:

- **Oncotype DX®** (a 21-gene RT-PCR assay; Genomic Health)
- **Oncotype DX® Breast DCIS Score**
- **70-gene signature MammaPrint®** (also referred to as the “Amsterdam signature”; Agendia)
- **Mammostrat®** (Clariant Diagnostic Services)
- **Molecular Grade Index (Aviara MG)^SM, AviaraDx, Inc.)**
- **Breast Cancer Index^SM, a combination of the Molecular Grade Index (MGI) and the HOXB13:IL17BR Index (bioTheranostics)**
- **BreastOncPx™ (Breast Cancer Prognosis Gene Expression Assay; LabCorp)**
- **Prosigna™ (NanoString Technologies)**
- **NexCourse® Breast IHC4 (Geneoptix)**
- **BreastPRST™ (Signal Genetics)**
- **EndoPredict® (Myriad Genetics)**
- **BluePrint® (Agendia)**
- **TargetPrint® (Agendia)**

If these panels are more accurate than current conventional risk classifiers, they could be used to aid chemotherapy decision-making, where current guidelines do not strongly advocate its use, without negatively affecting disease-free and overall survival outcomes.

**Oncotype DX® Breast DCIS Score**, which uses a slightly different algorithm than the standard Oncotype DX® to calculate results, is marketed for patients with noninvasive, ductal carcinoma in situ (DCIS) to predict the 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.

Of note, gene expression profiling should not be ordered as a substitute for standard ER or progesterone receptor (PR) testing. Gene expression profiles to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy should only be ordered after surgery and subsequent pathology examination of the tumor have been completed. The test should be ordered in the context of a physician-patient discussion regarding risk preferences and when the test result will aid the patient in making decisions regarding chemotherapy.

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 the 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-like breast cancers are ER positive, basal-like breast cancers correlate best with ER, PR and HER2 negative (“triple negative”), and HER2 type with high expression of HER2.

At present, the 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 which classifies breast cancer into basal type, luminal type or ERBB2-type. The test is marketed as an additional stratification into a molecular subtype following risk assessment with MammaPrint®.
• TargetPrint® is a microarray-based gene expression test which 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®.

MEDICAL POLICY CRITERIA

Note: This policy does not address the identification of germ-line DNA alterations in genes (BRCA1 and BRCA2) to provide information on future risk of hereditary breast or ovarian cancer. BRCA1 and BRCA2 testing is addressed in a separate medical policy (see Cross References).

I. The use of Oncotype DX®, Breast Cancer IndexSM, or Endopredict® in women with primary breast cancer, stage I, II, or III (see Policy Guidelines), to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy may be considered medically necessary when all of the following criteria are met:

A. Individual has had excision of breast mass and full pathologic evaluation of the specimen has been completed (i.e., the test should not be ordered on a preliminary core biopsy).
B. Primary tumor size 0.6–1 cm with moderate/poor differentiation or unfavorable features, or tumor size of 1 cm or greater.

If there are 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 as treatment is based on the most aggressive lesion.

C. Hormone receptor positive (that is ER-positive or PR-positive)
D. HER2-negative
E. Negative lymph nodes (nodes with micrometastases of 2 mm or less in size are considered node negative)
F. The test result will aid the patient in making a decision regarding chemotherapy when chemotherapy is a therapeutic option.

II. Use of Oncotype DX®, Breast Cancer IndexSM, or Endopredict® to determine recurrence risk in patients with primary breast cancer who do not meet criteria I.A – I.F. above is considered not medically necessary.

III. Use of Oncotype DX®, Breast Cancer IndexSM, or Endopredict® to determine patient risk in patients with primary breast cancer who meet criteria I.A – I.F. above but who have already made the decision to undergo or forego chemotherapy is considered not medically necessary.

IV. All other uses of Oncotype DX®, Breast Cancer IndexSM, or Endopredict® are considered
investigational, including but not limited to:

A. Predicting response to specific chemotherapy regimens
B. Determining HER2 status
C. Use of the tests in patients with other than stage I, II, or III breast cancer (see Policy Guidelines)

V. All other assays of genetic expression in breast tumor tissue are considered investigational, including but not limited to:

A. 70-gene signature MammaPrint®
B. Mammostrat® Breast Cancer Test
C. Molecular Grade Index
D. BreastOncPx™
E. Prosigna™
F. NexCourse® Breast IHC4
G. BreastPRS™
H. Oncotype DX® Breast DCIS Score

VIII. The use of gene expression assays to molecularly subclassify breast cancer, including but not limited to BluePrint®, is considered investigational.

IX. The use of gene expression assays for quantitative assessment of ER, PR, and HER2 overexpression, including but not limited to TargetPrint® is considered investigational.

X. The use of gene expression assays in men with breast cancer is considered investigational.

POLICY GUIDELINES

Ductal carcinoma in situ (DCIS) is considered stage 0 breast cancer and is therefore addressed in criterion IV.C.

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review:

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutations being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
6. Medical records related to this genetic test
• History and physical exam
• Conventional testing and outcomes
• Conservative treatment provided, if any

SCIENTIFIC EVIDENCE[1]

This evidence review focuses on gene expression profiling (GEP) panels that have prognostic or predictive ability in individuals with early-stage, invasive breast cancer with known estrogen receptor (ER) and progesterone receptor (PR) and human epidermal growth factor receptor (HER2) status. The proposed clinical utility of these tests varies depending on the clinical context; specific areas of proposed clinical utility are discussed in this evidence review:

1. Prognosis in patients with node-negative, early-stage, HER2-negative invasive breast cancer who will receive adjuvant hormonal therapy for the purpose of determining whether patients can avoid adjuvant cytotoxic chemotherapy.

2. Prognosis in patients with node-positive (1-3 nodes), early stage, HER2-negative invasive breast cancer who will receive adjuvant hormonal therapy for the purpose of determining whether patients can avoid adjuvant cytotoxic chemotherapy.

3. Prognosis in patients with node-negative, early-stage, HER2-negative invasive breast cancer, receiving adjuvant hormonal therapy, who have survived without progression to five years post-diagnosis, for the purpose of determining whether patients should continue adjuvant hormonal therapy.

4. Prognosis in patients with ductal carcinoma in situ (DCIS) for the purpose of selecting patients for radiation therapy.

Randomized controlled trials (RCTs) comparing health outcomes in women with primary breast cancer, who are managed with versus without gene expression profiling assays, are necessary to reliably establish the clinical utility of these assays.

In 2014, the Blue Cross and Blue Shield Association (BCBSA) Technology Evaluation Center (TEC) addressed gene expression profiling in women with lymph node-negative breast cancer to select adjuvant chemotherapy, specifically the use of Oncotype DX®, MammaPrint®, the Breast Cancer IndexSM, and Prosigna™/PAM50 gene expression assay.[2] (Note: The 2014 TEC report does not address the use of gene expression profiling in women with lymph node-positive breast cancer to guide adjuvant chemotherapy.) The TEC Assessment concluded that the use of Oncotype DX® to assess the risk of recurrence and to determine if a patient should undergo adjuvant chemotherapy in women with unilateral, hormone receptor-positive, lymph node-negative breast cancer, who will receive hormonal therapy, met the BCBSA TEC criteria. The TEC assessment also concluded 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.

Earlier in 2014 the Agency for Healthcare Research and Quality (AHRQ) published a Technology Assessment of molecular pathology testing for the estimation of prognosis for common cancers, which included assessments of Oncotype DX® Breast and MammaPrint®.[3] AHRQ concluded that there was moderate evidence that Oncotype DX® Breast leads to changes in treatment decisions. The Technology Assessment stated:
Although the decision changes were observed in both directions for individual patients, studies consistently showed an overall shift to less-intensive treatment recommendations as a result of using Oncotype DX® Breast, with fewer recommendations for chemotherapy (and therefore less exposure to potential harms of chemotherapy; but studies did not follow patients to actually report on harms or to assess the overall balance of clinical benefits and harms).

AHRQ also concluded that there was insufficient evidence to determine the impact of MammaPrint® on treatment decisions and clinical utility, primarily due to unknown consistency and imprecision.

Oncotype DX® (Genomic Health, Inc.)

Description

Oncotype DX® is available only from the CLIA-licensed Genomic Health laboratory as a laboratory-developed service. The test has not been cleared by the FDA; to date, FDA clearance is not required, although this may change if and when the FDA draft In Vitro Diagnostic Multivariate Index Assay (IVD-MIA) guidelines are finalized and published. Genomic Health has expanded indications for Oncotype DX® to include all stage 2 diseases (tumor ≤2 cm with spread to axillary lymph nodes or 2-5 cm without lymph node involvement) and ductal carcinoma in situ (DCIS).

Results from the Oncotype DX® gene expression profile are combined into a recurrence score (RS). Tissue sampling, rather than technical performance of the assay, is likely to be the greatest source of variability in results. The Oncotype DX® assay was validated in studies using archived tumor samples from subsets of patients enrolled in published 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 estrogen receptor positive, most were HER2 negative, and in the case of at least one study, multifocal tumors were excluded.[4]

Oncotype DX® in Lymph Node-Negative Patients

As described above, the 2014 BCBSA TEC Assessment concluded that the following circumstance meets the TEC criteria: Use of Oncotype DX® to determine recurrence risk in women with unilateral, hormone receptor-positive, lymph node-negative breast cancer, who will receive hormonal therapy, and are deciding whether to undergo adjuvant chemotherapy.[2] In the AHRQ Technology Assessment described above, the 16 studies included in the assessment uniformly examined cohorts with hormone-receptor positive breast cancer, and most were limited to women with node-negative cancers.[3] The studies below support the BCBSA TEC Assessment recommendation.

Validation Studies in Lymph Node-Negative Patients

Studies have attempted to delineate the association between RS and recurrence risk in node-negative patients.[5-8] Results indicate strong, independent associations between Oncotype DX® RS results and distant disease recurrence or death from breast cancer.[7,9]

In secondary analyses of data published by Paik et al. 2004a,[6,7] patient risk levels were individually classified by conventional risk classifiers, and 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 are actually at lower risk of recurrence (average 7%-9% risk at 10 years; upper 95% confidence interval
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 that chemotherapy will not provide net benefit; outcomes are improved by avoiding chemotherapy toxicity.

Supportive evidence is provided by an additional study that evaluated Oncotype DX®. In another RCT, samples were obtained from ER-positive, node-negative breast cancer patients, who were either treated with tamoxifen or tamoxifen plus chemotherapy, and were tested by Oncotype DX®.\[4\] RS high-risk patients derived clear benefit from chemotherapy, whereas the average benefit for other patients was statistically not significant.

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

- Those receiving aromatase inhibitor (AI)-based hormonal 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.

- Those receiving anthracycline-based chemotherapy instead of CMF. The type of chemotherapy does not change the interpretation of the Oncotype DX® risk estimate. Additionally, a recent meta-analysis indicates that anthracyclines do not improve disease-free or overall survival in women with early HER2-negative breast cancer\[10\], and therefore may not be prescribed in this population.

- Lymph nodes with micrometastases are not considered positive for purposes of treatment recommendations.\[11\] Current practice largely involves a detailed histologic examination of sentinel lymph nodes allowing for the detection of micrometastases (< 2 mm in size). Those whose tumors are ER-positive or 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 stage breast cancer. Recent studies show that ER-negative, PR-positive patients also tend to benefit from hormonal therapy.\[12,13\] Studies documenting the low incidence (1%-4%) and instability (lack of reproducibility) of the ER-negative/PR-positive subtype\[14\] and the reduction in reports of this subtype with improved assay techniques\[15\] suggest that this subtype may represent a false-negative result.

Other Studies of Oncotype DX® in Lymph Node-Negative Patients

Several nonrandomized studies reporting on the use of the 21-gene assay in lymph-node negative patients have been published (described below).

In 2015, Sparano et al. reported 5-year survival results of a prospective trial of endocrine therapy alone in breast cancer patients with low recurrence scores.\[16\] Patients were assigned to receive endocrine therapy without chemotherapy if they had a recurrence score of 0-10. At 5-years follow-up, 1626 women with low recurrence scores were included in the analysis. In this patient population, the rate of invasive disease–free survival was 93.8% (95% CI 92.4 to 94.9), the rate of freedom from distant disease was 99.3% (95% CI 98.7 to 99.6), and the rate of freedom from recurrence of breast cancer at a distant or local–regional site was 98.7% (95% CI 97.9 to 99.2).
Toi et al. confirmed the clinical validity of the 21-gene profile in a Japanese population of ER-positive, lymph node-negative patients with similar results for risk of distant recurrence in the three RS categories as in the original validation studies.\textsuperscript{[17]}

Mamounas et al. investigated the association between RS and risk for locoregional recurrence (LRR), as opposed to distant recurrence, in patients from the two NSABP trials. For 895 tamoxifen-treated patients, the 10-year Kaplan-Meier estimate of LRR was 4.3\% (95\% CI: 2.3-6.3\%) for patients with a low RS (<18), 7.2\% (3.4-11.0\%) for those with intermediate RS (18-30); and 15.8\% (10.4- 21.2\%) for those with a high RS (>30). LRR results were higher for those in all RS groups treated with placebo, and lower for those in all RS groups treated with tamoxifen and chemotherapy. Thus, RS was a significant and independent predictor of LRR along with initial treatment type.\textsuperscript{[18]}

Tzeng et al. examined how women receive and incorporate the results of their 21-gene profiles using mailed survey and chart review.\textsuperscript{[19]} About two-thirds of women indicated they understood most or all of the information regarding their recurrence risk based on their test results. The majority who experienced test-related distress had intermediate or high estimated recurrence risks by the 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 lived in urban areas.

Tang et al. compared the prognostic and predictive utility of RS and Adjuvant! in the NSABP B-14 and B-20 trial patients.\textsuperscript{[20]} An Adjuvant! Risk Index (RI) was fashioned with cutoff points allowing a patient risk distribution similar to that of the 21-gene RS. The results of the study demonstrated that the RS and Adjuvant! RI are independent prognostic factors of risk of distant recurrence; in addition, while RS was significantly predictive of chemotherapy benefit, Adjuvant! was not.

Several studies have been published regarding the impact of RS results on chemotherapy recommendations by medical oncologists (studies described below).\textsuperscript{[21-27]} According to these studies, comparing recommendations made prior to and revised after knowledge of RS results show that decisions change in about 25-40\% of patients, most often from endocrine therapy plus chemotherapy to endocrine therapy alone.

In 2013, Carlson et al. conducted a systematic review of studies of Oncotype DX\textsuperscript{®} used to inform actual adjuvant chemotherapy decisions in ER-positive, lymph node-negative patients with early-stage breast cancer.\textsuperscript{[28]} In eight identified studies (total N=1437), Oncotype DX\textsuperscript{®} RS changed the chemotherapy recommendation based on clinical-pathological factors in 33\% of patients. Compared with Oncotype DX\textsuperscript{®} high risk patients, low risk patients were statistically more likely to follow Oncotype DX\textsuperscript{®}-directed treatment (relative risk [RR], 1.07 [95\% CI: 1.01-1.14]).

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 with women with Oncotype DX\textsuperscript{®}-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).\textsuperscript{[27]}
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 (QOL), and satisfaction with choice of treatment. However, this study did not address the issue of whether results were described using a similar format for all patients so that they all had as close to the same information base as possible.[21]

The results of these studies may be suggestive of clinical utility because more patients avoid the toxicity of chemotherapy; however, there are no patient outcomes evaluated in these studies, and outcomes are assumed based on the original assay clinical validity evidence. 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.

**Oncotype DX® in Lymph Node-Positive Patients**

**Systematic Review**

In a recent systematic review partly funded by Genomic Health, Brufsky[29] assessed several published clinical trials and international congress abstracts, which evaluated the 21-gene breast cancer profiling assay (using RT-PCR technology) in patients with ER + and N+ early-stage breast cancer. Study results suggested that the Recurrence Score (RS) is an independent predictor of disease-free survival, overall survival, and distant recurrence-free survival. Overall, these studies showed that in 26% of 51% of N+ cases, physicians used results of the Recurrence Score (RS) assay to reassess patient status and ultimately change their treatment recommendations. In 60% to 66% of node-negative and node-positive cases, changes in treatment recommendations resulted in the elimination of chemotherapy.

Despite some favorable results of clinical utility, accompanied by author recommendations supporting the use of RS, the overall quality of the review was hampered by several methodological limitations. For example, study authors did not clearly report the systematic methodology used to conduct the literature search, such as details of the literature search criteria or inclusion and exclusion criteria used during the study selection process. In addition, they did not report assessing the quality of the individual clinical studies nor the body of evidence. Authors included abstracts presented at international congresses for detailed evidence review; however, results of these abstracts have yet to be accepted and published by a peer-reviewed journal. Hence, these various limitations substantially weaken the confidence in the findings that support clinical utility of the 21-gene assay in women with node-positive, early-stage breast cancer.

**Clinical Studies of Oncotype DX® in Lymph Node-Positive Patients**

Several individual clinical studies were identified during an independent literature review of the peer-reviewed published literature are included for review. The following four individual studies discussed below were also included in the Brufsky systematic review described above. [30-34]

Oratz et al. surveyed oncologists who were already ordering the 21-gene profile for lymph node-positive patients to determine the effect of the assay results on treatment recommendations; approximately half changed their recommendations after receiving RS results, with 33% recommending endocrine therapy alone instead of endocrine plus chemotherapy.[33] The majority of the 160 respondents (16% response rate) reported being satisfied with the data supporting the use of the assay in node-positive disease. However, medical oncologists who treat breast cancer patients were not surveyed in general, only those who were already using the assay, thus skewing the results. Finally, no outcomes were reported so the
study provided no firm evidence of clinical utility in this population.

Dowsett et al. reported on the Oncotype DX® recurrence score (RS) validation study in a subpopulation of the Arimidex, Tamoxifen, Alone or in Combination (ATAC) study. The authors evaluated patients who had hormone receptor-positive disease, who received single-agent therapy, and whose tissue blocks were available for analysis. Both node-positive (n=872) and node-negative (n=306) patients were included in the analysis. Of 306 node-positive patients, 243 had one to three involved nodes, and 63 patients had four or more; these were not analyzed separately. However, there is a graphic depicting outcomes of patients with 1-3 nodes and >=4 nodes showing that outcomes of patients with one to three nodes are intermediate between patients without nodes and those with >=4 nodes. Rates of distant recurrence at nine years were 17% (95% CI: 12-24%), 28% (95% CI: 20-39%), and 49% (95% CI: 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.” In addition, the HER2 status was not taken into consideration in this retrospective analysis.

Albain et al. reported that the Oncotype DX® assay may provide predictive information for chemotherapy benefit in addition to tamoxifen in post-menopausal axillary lymph node positive ER-positive breast cancer patients. They evaluated 8814 samples from the Southwest Oncology Group Trial (SOGT), in which outcomes from randomized node-positive, ER-positive patients treated with tamoxifen for five years were compared with those treated with cyclophosphamide, doxorubicin, fluorouracil (CAF) chemotherapy followed by tamoxifen (CAF-T) for five 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 two 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 was also predictive of response to chemotherapy. DFS at 10 years in the low risk group receiving tamoxifen was 60%.

A multivariable analysis of RS interaction with DFS, adjusted for number of positive nodes, was significant for the first five 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. This was a retrospective subset analysis from a single randomized clinical trial. The patient selection for assay use remains controversial and additional studies are necessary before it is possible to confidently withhold previously recommended chemotherapy from lymph node-positive breast cancer patients with low/intermediate RS results.

Goldstein and colleagues reported on use of the 21-gene assay in a sample of patients, 44% of whom had one to three positive nodes, and all of whom were treated with chemohormonal therapy. This study found that RS was a highly-significant predictor of recurrence for node-negative and node-positive disease. The authors concluded that both RS and standard clinical and pathological features contributed significantly and independently to recurrence prediction. The findings also suggested that it may be possible to withhold adjuvant chemotherapy from patients with one to three positive axillary lymph nodes with low RS. However, as the authors noted, because there was no arm without chemotherapy treatment, it was not possible to directly evaluate whether the excellent outcome in those with low RS was related to good prognosis, chemotherapy benefit, or both. Therefore, additional, properly designed
studies are needed to support this indication.

The following two studies were included in the 2014 AHRQ Technology assessment.

de Boer et al. evaluated the impact of the Oncotype DX® recurrence score on treatment decisions in an uncontrolled trial of 151 early stage women with node-negative (n=101) and node-positive (0-3) (n=50) breast cancer.\[35\] Based on assay results, initial treatment decisions were revised in 24% of node-negative patients and 26% of node-positive patients.

Eiermann et al. evaluated the 21-gene recurrence score assay in 366 women with node-negative (n=244) and node-positive (0-3) (n=5) breast cancer.\[34\] Based on assay results, initial treatment decisions were revised in 33% of all patients (30% in node-negative; 39% in node-positive). Before the assay, adjuvant chemotherapy was recommended in 57% of women, while assay test results led to 54% of patients foregoing adjuvant chemotherapy. Knowledge of RS impacted both physicians’ and patients’ decisions regarding adjuvant chemotherapy that resulted in a significant reduction in adjuvant chemotherapy administration.

The following individual clinical studies were also identified during the independent literature search but these studies were not included in the 2014 Brufsky review or the AHRQ Technology assessment. In 2016, Gluz et al. reported on a prospective study designed to evaluate outcomes of patients who are selected to avoid chemotherapy based on their RS score.\[36\] This study included patients with positive nodes. The sample size of patients with 1-3 positive nodes is 930, but the size of the sample followed for long term outcome is uncertain. Chemotherapy was deferred in patients who had RS < 12. The three-year DFS for patients with one to three positive nodes with RS< 12 was 97.9%. The three-year DFS for patients with negative nodes was 98.6%. Although DFS is similar between node-positive and node-negative patients at three years, the number of events is very small at this time point (eight total events) and follow-up is still early.

Ueno et al.\[37\] conducted a small prospective study to evaluate the association between the Oncotype DX® RS from core biopsy and resection samples, and individual clinical response to neoadjuvant endocrine therapy, in postmenopausal women with node-positive and node-negative breast cancer (n=64). Study authors used archived tumor tissues from a previous study. Results of the assay and clinical response at baseline were compared with the same outcomes in patients with low assay result (<18) and patients with high assay result (≥31). Inclusion criteria were as follows: 55-75 years; ER+ and stage II or IIIa invasive breast cancer (T2-3, N0-2, M0). Treatment was exemestane (25 mg/day) for 16 weeks, with a possibility of an 8-week extension based on clinical response. The clinical response rate in patients with low RS (19/32, 59.4 %) was significantly higher than patients with high RS (3/15, 20.0 %) (P = 0.015). Additional sub-analysis showed that patients with low RS had a significantly greater percentage of tumor reduction (nearly 32%) compared with patients with high RS who had an average tumor reduction of 12.5% (P=0.045). Rates of breast conserving surgery among the three groups were as follows: low RS (nearly 91%); intermediate RS (76.5%), and high RS (nearly 47%). The odds ratio (OR) for breast conserving surgery between the high and low RS groups was 0.91 (95% CI: 0.019-0.432; P=0.003). Study authors concluded that RS was predictive of the clinical response to neoadjuvant chemotherapy in postmenopausal women. This study was hampered by a few limitations, including its use of historical controls, small sample size, and lack of assessment of lymph node response following neoadjuvant endocrine treatment.

Markopoulos et al. reported findings from the analysis of 106 women with ER-positive, HER2-negative early breast cancer for whom Oncotype DX® was performed in order to determine whether hormonal
therapy only or chemotherapy plus hormonal therapy was the optimal adjuvant treatment. However, the study had a retrospective design and it is not clear whether all patients in this study had node-positive status.

Joh et al. evaluated the impact of Oncotype DX® RS on chemotherapy recommendations and compared the estimated recurrence risk predicted by oncologists to RS. In the analysis, 154 women with ER-positive early stage breast cancer and available RS were considered. They report that oncologists tended to overestimate risk of recurrence and that 24.9% treatment plans were changed as a result of RS data. However, the study did not report breast-cancer related health outcomes in the study participants.

Although the previously described studies all demonstrate that RS stratifies patients with positive nodes into categories of patients with different risks of recurrence, the rates of recurrence shown in node positive patients are consistently higher than patients without positive nodes. The one study showing similar risks of recurrence has low number of events and reports outcomes at three years. It is not certain that the risk of recurrent disease is low enough to consider avoiding chemotherapy. The finding of a treatment interaction may not be robust enough to appropriately defer chemotherapy in patients who have higher recurrence risks than patients with node-negative disease.

Additional Applications of Oncotype DX®

In June, 2008, Genomic Health announced that results of Oncotype DX® tests would include not only the overall test results, but also the results of the quantitative ER and PR tests that are included in the Oncotype DX® panel. This is based on a study (published in May 2008) that compared the Oncotype DX® ER and PR results with traditional immunohistochemistry (IHC) results. The study reported high concordance between the two assays (90% or better), but that quantitative ER by Oncotype DX® was more strongly associated with disease recurrence than the IHC results. However, ER and PR analyses are 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. in 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. Although the HER2 component of the 21-gene assay has been shown to strongly correlate with FISH results, the 21-gene assay should not be ordered to determine or confirm HER2.

Clinical Practice Guidelines

National Comprehensive Cancer Network®

NCCN guidelines for breast cancer (version 2.2016) state that the Oncotype DX® assay be considered in hormone receptor positive, HER2 negative disease when the tumor is >0.5cm in the following stages: pT1, pT2, or pT3; and pN0 or pN1mi (≤2mm axillary node metastasis) (category 2A). In addition, the guidelines address node-positive disease, and state the following: “The 21-gene RT-PCR assay
recurrence score can be considered in select patients with one to three ipsilateral axillary lymph nodes to guide the addition of combination chemotherapy to standard hormone therapy. A retrospective analysis of a prospective randomized controlled trial suggests that the test is predictive in this group similar to its performance in node-negative disease.”

Albain et al. (2010) is the retrospective analysis cited by the NCCN guideline, and included for discussion in this policy.[30] Study results showed that patients with high RS scores appeared to achieve greater benefit from the addition of chemotherapy than patients with low RS scores, irrespective of the total number of affected lymph nodes. In the multivariate analysis of RS interaction with disease free survival, adjusted for number of positive nodes, was significant for the first five years of follow-up (p=0.029) and remained significant after adjusting for age, race, tumor size, PR 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.

American Society of Clinical Oncology (ASCO)[43]

ASCO 2016 guidelines on the use of biomarkers to guide decisions on therapy for women with early-stage invasive breast cancer recommends the use of the Oncotype DX® test as one of several tests that may be used women with ER/PgR-positive, HER2-negative, node-negative breast cancer. These recommendations are considered strong and are based on high quality evidence.

In patients with node-positive breast cancer, ASCO recommends against the use of this test, citing that “patients with node-positive disease but low RS have a worse prognosis than patients with node-negative, low RS disease”. The panel believes that because widespread use of adjuvant chemotherapy has had such a profound effect on reducing breast cancer mortality, that clinicians must take a cautious approach to withholding it from patients with node-positive disease.

U.S. Preventative Services Task Force (USPSTF)[44]

According to the USPSTF gene expression testing and other prognostic tests (e.g., immunohistochemistry) of breast cancer tumor tissue is not a preventive service.

Patients with Ductal Carcinoma in situ (DCIS)

Ductal carcinoma in situ (DCIS) is the presence of abnormal cells inside a milk duct in the breast. DCIS is considered the earliest forms of breast cancer and is noninvasive. DCIS requires treatment to prevent the condition from becoming invasive and most women diagnosed with DCIS are effectively treated with breast-conserving surgery and radiation. DCIS diagnosis accounts for about 20% of all newly diagnosed invasive plus noninvasive breast tumors. Recommended treatment is lumpectomy with or without radiation treatment; post-surgical tamoxifen treatment is recommended for ER-positive DCIS, especially if excision alone is used. The overall rate recurrence following DCIS diagnosis is less than 30% and usually occurs within five to 10 years after initial diagnosis.

The Oncotype DX® DCIS test uses information from 12 of the 21 genes assayed in the standard Oncotype DX® test for early breast cancer. Scaling and category cut-points are based on an analysis of DCIS Score results from a separate cohort of patients with DCIS; this study has not yet been published and is available only as a meeting abstract.[45]

In a 2015 retrospective analysis, Rakovitch et al. 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: 1.43-3.22). 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: 10-17), 33% (95% CI: 24-45), and 28% (95% CI: 20-38), respectively. Corresponding 10-year estimates for DCIS recurrence (5% [95% CI: 3-9]; 14% [95% CI: 8-24]; 14% [95% CI: 9-22], respectively) and for invasive breast cancer recurrence (8% [95% CI: 6-12]; 21% [95% CI: 13-33]; 16% [95% CI: 9-25], 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.

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). Oncotype DX® DCIS Score was significantly associated with recurrence outcomes (HR: 2.31 [95% CI: 1.15-4.49]; 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. The standard Oncotype DX® Score for early breast cancer was not associated with DCIS recurrence outcomes.

Although the DCIS score successfully stratifies patients into groups with different outcomes, it is unclear whether estimated recurrence risks for patients classified as low risk are low enough or estimated with sufficient precision to meaningfully affect the decision to have or forego radiotherapy.

Conclusion

These studies address the development of the Oncotype DX® DCIS Score and the clinical validity (statistical association of the test result with recurrence outcomes). Evidence for the clinical utility of Oncotype DX® DCIS is limited, and it is still uncertain if women are better categorized according to their recurrence risk using the Oncotype DX® DCIS Score compared with standard clinical indicators of risk.

Oncotype DX® in Men

Researchers have focused relatively little attention on male breast cancer compared with female breast cancer. While only 0.5%–1% of all breast cancers in the United States occur in men, approximately 2000 men are diagnosed with breast cancer annually. There are several risk factors for breast cancer in men, including age and family history. Family history is relevant for both sexes, and BRCA2 mutations and rearrangements play a particularly prominent role in male breast cancer. A total of 5% to 10% of men with BRCA2 mutations (and a smaller proportion of those with BRCA1 mutations) eventually develop breast cancer.

Evidence of the use of Oncotype DX® in men is limited to a single case study and one non-randomized study that includes one male patient out of 29 patients. Missing and conflicting data from retrospective registry studies limit definitive conclusions about grade and HER2 status of male breast cancers.
In a recent systematic review, relevant published data regarding risk factors, biological characteristics, presentation and prognosis, appropriate evaluation and treatment, and survivorship issues in male breast cancer patients were presented.\textsuperscript{[48]} BRCA2 mutations, age, conditions that alter the estrogen/androgen ratio, and radiation were proven risk factors. Authors concluded disease biology is distinct in men, but diagnostic approaches and treatments for men are generally extrapolated from those in women due to inadequate research in men. In addition, authors suggested survivorship issues in men may include sexual and hormonal side-effects of endocrine therapies as well as unique psychosocial impacts of the disease. Authors further concluded that further research is needed to address gaps in knowledge pertaining to care of male breast cancer patients and survivors. Oncotype DX® utilization is not addressed in the article.

\textbf{MammaPrint®}

\textbf{Description}

MammaPrint® has received 510(k) clearance for marketing by the FDA as a prognostic test for women younger than 61 years with ER-positive or ER-negative, lymph node-negative breast cancer. It is approved to assist in categorizing these breast cancer patients into high versus low risk for recurrence, but it is not approved for predicting benefit from adjuvant chemotherapy.

\textbf{Technology Assessments}

Based on the 2014 BCBSA TEC report, MammaPrint® does not meet TEC criteria in women with unilateral, hormone receptor-positive, lymph node-negative breast cancer who will receive hormonal therapy.\textsuperscript{[2]}

Based on the 2014 AHRQ Technology Assessment, there was insufficient evidence to determine the impact of MammaPrint® on treatment decisions and clinical utility, primarily due to unknown consistency and imprecision.\textsuperscript{[3]}

\textbf{Other Studies of MammaPrint®}

A prospective comparative phase III study published in 2016 enrolled 6,693 women with early-stage breast cancer and assessed their genomic risk using MammaPrint® and their clinical risk using a modified version of Adjuvant! Online for cancer recurrence.\textsuperscript{[50]} Women with low risk by both indicators did not receive chemotherapy, women with high risk by both indicators did receive chemotherapy, and when the risk indicators did not agree, the use of chemotherapy was randomized, based on either the clinical or the genomic risk. Due to a change in MammaPrint® reagents, there was a temporary shift in the risk calculation that lasted nearly eight months. Because of this, 162 patients who had been identified as being at high genomic risk were subsequently reclassified as having low genomic risk; 28 of these patients received chemotherapy prior to the correction, while the other 113 patients had their designations corrected. The primary endpoint for the study was a noninferiority outcome of 5-year metastasis free survival rate in one cohort of the study population: those with high clinical risk and low genomic risk who did not receive chemotherapy. Secondary analyses included outcome comparisons in patients in discordant risk groups between those who did and did not receive chemotherapy, outcome comparisons in all patients for whom chemotherapy was recommended by only one risk type, and calculation of the overall percentage of patients that would be assigned to chemotherapy based on either risk determination.
In this study, the median age of participants was 55 years (range: 23-71), 79% had node-negative disease, 88.4% had ER/PR-positive disease, and 9.5% had HER2-positive disease. The clinical and genomic risks were discordant in 2,147 patients. There were 1,550 patients with high clinical risk and low genomic risk (as determined by Mammaprint®), and the 5-year rate of survival without distant metastasis among those in this group who did not receive chemotherapy was 94.7% (95% CI: 92.5-96.2), while this rate was 95.5% in those who did receive chemotherapy (approximate difference of 1.5%). This difference was not adequately powered to reach statistical significance. Based on these results, the authors concluded that chemotherapy could be avoided in the approximately 46% of high-clinical risk breast cancers that are determined to be low genomic risk using Mammaprint®. The outcomes for patients at low clinical risk but high genomic risk who had chemotherapy were not meaningfully different than for those who did not have chemotherapy, so the information from the genomic risk test was not useful in those populations. While these results show potential for Mammaprint® to be useful in guiding adjuvant therapy decisions in the setting of high clinical risk for cancer recurrence, the study is limited by the heterogeneity of the patients included and the follow-up time of five years. Future results of this ongoing study will be valuable for assessing the overall clinical utility of this test.

In 2014, to assess the impact of MammaPrint® on treatment decision-making, Cusumano et al. distributed clinical information on 194 patients to multidisciplinary teams initially without and then with MammaPrint® gene signatures.[51] 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.

Ahn et al. investigated the use of MammaPrint® to further risk-stratify 82 ER-negative patients (56% lymph node-negative) who had Oncotype DX® intermediate risk scores.[52] Although MammaPrint® risk classification was significantly associated with 10-year overall survival in multivariate analysis (log-rank test, p=0.013), this result was confounded by receipt of adjuvant chemotherapy, which also was significantly associated with overall survival (log-rank test, p=0.024).

In 2014, Sapino et al published a validation study of MammaPrint® using formalin-fixed, paraffin-embedded (FFPE) tissue.[53] In a validation set of 221 tumor samples, concordance of FFPE and frozen tissue low- and high-risk classification was 91.5% (95% CI: 86.9-94.5). Concordance of repeat analyses of the same tumor was 96%, and inter-laboratory reproducibility (i.e., between labs in the Netherlands and in California) was 96%.

The Microarray Prognostics in Breast Cancer (RASTER) study, published in 2013, was designed to assess feasibility of implementation and impact on treatment decisions of the MammaPrint® 70-gene signature, as well as recurrence outcomes.[54] The study followed 427 node-negative, early-stage breast cancer patients who had a 70-gene signature (MammaPrint®, which was available to help direct post-surgery treatment decisions, and which was compared to Adjuvant! Online. All of the patients were aged 18-61 years old 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 estimates between MammaPrint® and AOL occurred in 38% of the cases (161/427). Most discordant cases were MammaPrint® low-risk and AOL high-risk (124/427= 29%), whereas 37 cases (37/427=9%) had a high-risk MammaPrint® and a low-risk AOL estimation. Use of MammaPrint® reduced the proportion of high-risk patients as classified by AOL by 20% (87/427).
5-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.

The results suggest that MammaPrint® is a better prognostic classifier than standard clinical and pathological classifiers. However, there are several limitations in the study design. The patient numbers were low and event numbers very low, making interpretation of the results difficult. The actual treatment decisions that were made were based on restrictive Dutch guidelines from 2004 and patients’ and doctors’ preferences. Additionally, the adjuvant online risk estimates were based on 10-year outcomes, whereas the RASTER outcomes were at five years. Since most clinical relapses in lymph node negative, ER positive breast cancers do not occur until five or even 10 years after diagnosis, with or without the use of adjuvant therapy, the study data should be considered not yet mature.

In 2013, Saghatchian et al. evaluated MammaPrint® signatures of frozen tumor samples from patients who had 4-9 positive lymph nodes.[55] 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 eight 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.

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 recurrence free survival.[56] Despite having locally advanced disease, patients with 70-gene low-risk profiles tended not to respond to chemotherapy and to have good short-term recurrence free survival. However, there is only three years of follow-up, and the number of low risk patients was small.

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.[57] Only 27 patients were classified as low risk by MammaPrint®, but distant metastasis-free survival at 10 years was 100%. For the 73 patients classified as high risk, distant metastasis-free survival at 10 years was about 90%, but there was no statistically significant difference in survival between the low- and high-risk groups. The patients studied were heterogeneous in terms of ER-positivity (73%), hormonal therapy (25%), and chemotherapy (23%); subpopulations were too small for separate evaluation of outcomes.

One small (n=123) study of lymph node-negative patients younger than 55 years, 76% with ER-positive tumors, who received variable treatment for early-stage breast cancer, reported that the 70-gene signature was significant in multivariate analyses for prognosis.[58] However, the small study size and small number of events precludes an adequate statistical analysis.[59] This study also updated results of the node-negative population from the validation study, reporting significantly different outcomes for good and poor gene signature prognosis groups, but estimates were very wide due to small numbers and a receiver operating characteristic (ROC) analysis also showed overlapping confidence intervals.[60]

Mook et al studied 241 node-positive patients with primarily ER-positive, HER2-negative tumors treated variably.[61] The 70-gene signature was a significant predictor of outcome overall and in individual treatment groups, but estimates had wide confidence intervals due to small numbers. Classification of patients by Adjuvant! Online, then recategorization by MammaPrint® showed
additional discrimination of outcomes by the gene signature, but results were confounded by heterogeneous patient treatment. This study also updated the results of 106 patients with 1-3 positive nodes from the validation study,[19] reporting 98% (95% CI: 94-100%) 10-year breast cancer-specific survival for good prognosis signatures vs. 64% (95% CI: 52-76%) for poor prognosis signatures; adjusted HR 3.63 (95% CI: 0.88–14.96), p=0.07. Based on these results, the ongoing MINDACT trial of MammaPrint® is being 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 the risk of recurrence according to MammaPrint®.[62]

A study of patients with heterogeneous tumors receiving neoadjuvant treatment reported preliminary data that patients with good prognosis signatures did not benefit from neoadjuvant treatment and were less likely to relapse.[58]

Other studies of MammaPrint® have been published, however the studies are generally small and/or retrospective or pooled re-analyses of subgroups from previously published retrospective studies.[63-70] In addition, several studies assessing the impact of MammaPrint® testing on treatment decision-making did not include survival or recurrence outcomes and are therefore considered uninformative for assessing clinical utility of MammaPrint®.[71,72]

Additional Applications of MammaPrint®

In 2014 Drukker et al applied MammaPrint® to 1053 tumor specimens from 1848 patients enrolled in eight previous MammaPrint® studies in order to examine the ability of gene expression tests to provide risk information for locoregional recurrence.[73] 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 nine years, estimated 10-year locoregional recurrence risk was 13% (95% CI: 10-16) for 492 patients categorized as MammaPrint® high-risk versus 6% (95% CI: 4-9) for 561 MammaPrint® low-risk patients. This association was observed during the first five years after diagnosis, but not during years five to 10. Recurrence stratified by MammaPrint® risk class was not predictive of treatment response.

Clinical Practice Guidelines


Current ASCO[74] guidelines recommend against the use of MammaPrint® to decide whether a patient should receive adjuvant chemotherapy, regardless of hormone receptor or node status, stating that the assay cannot identify a group of patients for whom chemotherapy is either not required or not effective.

Conclusion

The majority of MammaPrint® studies, including early validation studies, suffered from confounding due to heterogeneous patient samples. Subsequent pooled re-analyses of subpopulations controlled for one variable (e.g., nodal status), but confounding remained from other variables (e.g., treatment heterogeneity, ER status, HER2 status). It is therefore difficult to estimate outcomes for the patients of interest: ER+, HER-patients not receiving chemotherapy. Studies tended to be small and results had
wide confidence intervals that could not rule out too much risk to consider withholding chemotherapy. 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 published RASTER (Microarray Prognostics in Breast Cancer) study and the randomized trial by Cardoso et al. represented improved study designs, and results of these studies suggested that MammaPrint® may accurately re-classify early, node-negative breast cancer patients classified as high risk for recurrence by clinical and pathologic variables to low risk, such that chemotherapy may be avoided. However, patient numbers and events are too low for firm conclusions, and follow-up is not yet sufficiently mature.

**Breast Cancer Index** SM (BCI)

**Description**

The Breast Cancer Index SM is a simultaneous assessment of the HOXB13:IL17BR (H/I) ratio and the MGI (Molecular Grade Index). The H/I ratio indicates estrogen-mediated signaling; MGI assesses tumor grade by measuring the expression of five 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%–25%, likely too high for most patients and physicians to consider forgoing chemotherapy. Studies of the combination Breast Cancer Index SM are reviewed below.

**Technology Assessments**

The Breast Cancer Index SM does not meet TEC criteria based on the 2014 BCBSA report to determine recurrence risk in women with unilateral, hormone receptor-positive, lymph node-negative breast cancer.

**Other Studies of Breast Cancer Index SM**

A 2016 retrospective study by Sgroi et al. evaluated the use of the BCI in samples from the NCIC MA.14 clinical trial of tamoxifen alone vs. tamoxifen plus octreotide in postmenopausal women with early breast cancer. A total of 292 samples from banked tumor blocks were assayed: 146 from each treatment arm. BCI was categorized as high-risk (BCI ≥6.4), intermediate risk (5 ≤ BCI < 6.4), and low risk (BCI < 5). These risk groups were associated with adjusted 10-year relapse-free survival, which was 87.5% in the low-risk group, 83.9% in the intermediate-risk group, and 74.7% in the high-risk group. There was no significant interaction between BCI and treatment group. Because most LN-positive patients received chemotherapy, the prognostic utility of BCI could not be assessed for those patients.

In 2013, Sgroi et al. examined 665 lymph node-negative, ER-positive, postmenopausal women receiving endocrine therapy but no chemotherapy in the ATAC trial. In this group, approximately 10% of samples were HER2+. Two versions of the Breast Cancer Index (BCI) score were generated in the study: the BCI-C, based on cubic combinations of the variables, and the BCI-L, based on linear combinations of the variables. The BCI-L, which is the model used in the development studies by Zhang et al. described above and represents the commercial version of the BCI, was more effective than the BCI-C at risk discrimination. The overall 10-year distant recurrence rates for the BCI-L low, intermediate, and high risk groups were 4.8% (95% CI: 3.0% to 7.6%), 18.3% (95% CI: 12.7% to 25.8%), and 29.0% (95% CI:
21.1% to 39.1%), respectively. For patients in the low- and intermediate-risk groups, 10-year distant recurrence risks were similar, regardless of endocrine treatment (tamoxifen, anastrozole, or both).[76] In the high-risk group, recurrence risk was lowest (22%) for patients taking anastrozole only and highest for patients taking tamoxifen only (37%), although these groups were small (54 and 55 patients, respectively).

In 2013, Zhang et al. evaluated a continuous risk model derived from the H/I ratio and MGI in tumor samples from the same RCT used by Jerevall et al (the Stockholm tamoxifen cohort; n=317), along with additional samples from a multi-institutional registry of ER-positive, lymph node-negative patients (n=358), 32% of whom received adjuvant chemotherapy.[77] An optimized continuous recurrence risk model, the Breast Cancer IndexSM model, was built using patients from the untreated arm of the Stockholm cohort as a training set. Samples from the endocrine therapy arm of the Stockholm trial and from the multi-center registry were used for the validation studies. The Stockholm validation set included 7% HER2+ samples and the multi-center registry included 12% HER2+ samples. The overall 10-year distant recurrence rates for the BCI low, intermediate, and high risk groups in the Stockholm cohort were 4.8% (95% CI: 1.7% to 7.8%), 11.7% (95% CI: 3.1% to 19.5%), and 21.1% (95% CI: 15.3% to 32.0%), respectively, while the 10-year distant recurrent rates for these groups in the multi-center registry were 6.6% (95% CI: 2.9% to 10%), 23.3% (95% CI: 12.3% to 33%), and 35.8% (95% CI: 24.5% to 45.5%), respectively.

Jerevall et al. combined the H/I Ratio and MGI into a continuous risk model using 314 ER-positive, node-negative post-menopausal patients from the tamoxifen-only arm of a randomized controlled trial.[78] The continuous model was also used to categorize patients into groups of low, intermediate, and high-risk. 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 recurrence or death 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. There are no reclassification studies of comparison with conventional risk classifiers; thus, clinical utility in a population likely to be treated with tamoxifen is unclear.

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.[79] 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%.

Clinical Practice Guidelines


Current ASCO[74] guidelines on the use of biomarkers to guide decisions on adjuvant therapy for women with early-stage invasive breast cancer state that the Breast Cancer IndexSM test is one of several tests that may be used in women with ER/PgR-positive, HER2-negative, node-negative breast cancer. The
strength of this recommendation is considered moderate and based on intermediate quality evidence by the guideline authors.

**The Molecular Grade Index (Aviara MGISM)**

**Description**

The Molecular Grade Index (Aviara MGISM) assay is intended to measure tumor grade using the expression of five cell cycle genes and to provide prognostic information in ER-positive patients regardless of nodal status.

**Studies of Aviara MGISM**

Ma et al. evaluated MGI along with Aviara H/ISM in a total of 733 patients.\(^8\) High MGI was associated with significantly worse outcome only in patients with high Aviara H/ISM and vice versa. Both assays are offered separately; the utility of MGI alone is unclear. There are no reclassification studies of comparison with conventional risk classifiers.

**Clinical Practice Guidelines**

Currently, neither NCCN nor ASCO address the use of the Molecular Grade Index (Aviara MGISM) as an option when evaluating breast cancer patients for risk of recurrence.

**Mammostrat®**

**Description**

Mammostrat® is an IHC test intended to evaluate risk of breast cancer recurrence in postmenopausal, node negative, ER-positive breast cancer patients who will receive hormonal therapy and are considering adjuvant chemotherapy. The test employs five monoclonal antibodies to detect gene expression of proteins involved in various aspects of cell proliferation and differentiation and a proprietary diagnostic algorithm to classify patients into high-, moderate-, or low-risk categories.

**Studies of Mammostrat®**

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.\(^\text{[81]}\) Tumor samples from two 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 five 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 ($R^2$ statistic and index of discrimination) indicated that after five years (in the subset of patients who remained distant-recurrence free for at least five years, n=3920 [81%]), there was no evidence of an association between Mammostrat® scores and time to distant recurrence.

Bartlett et al. reported that Mammostrat® can act as an independent prognostic tool for ER-positive,
tamoxifen-treated breast cancer. However, this was a retrospective case series that included both node-positive and node-negative patients.[82]

Ross et al. examined the same trial samples used for Oncotype DX® validation (NSABP B-14 and B-20 trials) and reported 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.[83] Both low- and high-risk groups, but not moderate-risk groups, benefited significantly from chemotherapy treatment. A test for an interaction between chemotherapy and the risk group stratification was not significant (p = 0.13).

Ring et al. reported the development of the assay but provided no information on technical performance (analytic validity).[84] In an independent cohort, a multivariable model predicted 50%, 70%, and 87% 5-year disease-free survival for patients classified as high, moderate, and low prognostic risk, respectively, by the test results (p=0.0008).

There are no published Mammostrat® reclassification studies of comparison with conventional risk classifiers.

Clinical Practice Guidelines

Current NCCN guidelines do not address the use of Mammostrat® as an option when evaluating breast cancer patients for risk of recurrence.

Current ASCO[74] guidelines recommend against the use of Mammostrat®, stating that the group of patients considered low-risk by the assay had 10-year recurrence risks that were low.

BreastOncPx™

Description

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.

Studies of BreastOncPx™

Tutt et al. published information on the development and validation of the test.[85] 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 - 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. However, the MS was not added to Adjuvant! and was not compared to Adjuvant! alone. No reclassification analysis was conducted.

Clinical Practice Guidelines

Currently, neither NCCN nor ASCO address the use of BreastOncPx™ as an option when evaluating breast cancer patients for risk of recurrence.

**NexCourse® Breast IHC4**

**Description**

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.[86,87]

**Studies of NexCourse® Breast IHC4**

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 five years after diagnosis.[83] Measures of calibration and discrimination indicated that after five years, there was no evidence of an association between IHC4 scores and time to distant recurrence.

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.[88] Of these, 793 were node-negative and 59 were HER2-positive (but were not treated with trastuzumab). A prognostic model that combined the four 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.
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)[89]. 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 three 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.

Clinical Practice Guidelines

Current NCCN guidelines do not address the use of IHC4 as an option when evaluating breast cancer patients for risk of recurrence.

Current ASCO[74] guidelines recommend against the use of IHC4, stating that the test is not sufficiently reproducible, despite evidence of clinical utility.

**Prosigna™/ PAM50 Breast Cancer Intrinsic Subtype Classifier**

**Description**

PAM50 Breast Cancer Intrinsic Classifier, a qRT-PCR test based on a panel of 50 genes, was developed to identify the breast cancer intrinsic subtypes known as 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 their cancer. Prosigna™ evolved from the PAM50 test and uses NanoString’s nCounter platform[90] in place of qRT-PCR to assay 46 genes instead of the original 50.

**Technology Assessment**

The 2014 TEC Assessment reviewed development and validation studies of the PAM50 intrinsic subtype classifier and Prosigna™;[2] these studies are reviewed below. Only two studies of the marketed Prosigna™ test were identified, one of which reported analytic validity. A third study performed the commercial assay on 46 of the PAM50 genes, excluding one HER2-associated gene (GRB7) and three proliferation-associated genes (BIRC5 [also called Survivin], MYBL2, and 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 below.

**Studies of Prosigna™/PAM50 Breast Cancer Intrinsic Classifier**

Two studies published in 2015 presented combined analyses of pretreatment FFPE tumor specimens from ABCSG-8 and ATAC trial monotherapy arms (TransATAC).[91,92] 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).[91] Patients had hormone receptor-positive invasive breast cancer treated with only endocrine therapy (anastrozole or tamoxifen; no chemotherapy) for five years without recurrence. The majority of patients (74%) had node-negative disease (87% of patients with node-positive disease had one to three 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 (between five and 10 years) in node-negative patients were 2.3% (95% CI: 1.3-3.5), 8.5% (95% CI: 5.9-12.1), and 9.3% (95% CI: 5.5-15.5), respectively. In node-positive patients, estimated risks were 3.3% (95% CI: 1.2-8.6), 7.8% (95% CI: 4.4-13.8), and 20.9% (95% CI: 16.1-26.9) in low-, intermediate-, and high-risk groups, respectively. It is worth noting that prediction of 10-year survival contingent on five year survival without recurrence is not informative for treatment decisions at the time of diagnosis.

Liu et al. 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.[93] MA.21 was an international, phase three 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 recurrence-free survival (RFS), but categorical ROR was associated with neither RFS nor survival by treatment group (ie, neither prognostic nor predictive). Intrinsic subtypes were associated with RFS but were not predictive of treatment outcomes. The authors stated:[93]

“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.”

Cheang et al. determined PAM50 intrinsic subtypes for samples from a clinical trial randomizing premenopausal women with node-positive breast cancer to two different regimens of chemotherapy. The PAM50 intrinsic subtype for 476 tumors was correlated to RFS (p=0.0005) and overall survival (p<0.0001).[94] The HER2-enriched subgroup (22%) showed the greatest benefit from cyclophosphamide-epirubicin-fluorouracil (CEF) versus cyclophosphamide-methotrexate-fluorouracil (CMF), with absolute 5-year RFS and OS differences exceeding 20%. There was a less than 2% difference for non–HER2-enriched tumors (interaction test p=0.03 for RFS and 0.03 for survival). Within clinically defined HER2-positive tumors, 79% (72 of 91) were classified as the HER2-enriched subtype by gene expression, and this subset was associated with better response to CEF versus CMF (62% vs. 22%, p=0.0006). There was no significant difference in benefit from CEF versus CMF in basal-like tumors.

Martin et al. evaluated the impact of ROR on treatment decision making in patients with ER-positive,
HER2-negative, node-negative breast cancer.[95] Because survival or recurrence outcomes were not reported, the study is considered uninformative for assessing clinical utility of Prosigna™.

The following studies included in the 2014 TEC Assessment:

Gnant et al. evaluated FFPE tissue specimens from 543 patients in the ABCSG-8 and ATAC trials who had one to three positive lymph nodes.[92] 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.[88] Risk classification by CTS was compared with and without ROR in subsets of patients with one positive lymph node (n=331) and with two to three 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., discussed above. Among patients with one 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: 3.3-12.8), 15.5% (95% CI: 9.5-25.0), and 25.5% (95% CI: 17.5-36.0), 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 two or three 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% were categorized as high risk. The 10-year distant RFS estimates were 12.5% (95% CI: 6.6-22.8) and 33.7% (95% CI: 25.5-43.8), 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.

Nielsen et al. (2014) assessed the analytical performance of Prosigna™ using the proprietary nCounter Analysis System (NanoString Technologies) at NanoString Technologies and two other laboratories.[97] 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 five tumor RNA samples 36 times at the three labs. Standard deviation across labs was less than one ROR unit on the 0-100 ROR scale. Reproducibility was measured by testing 43 FFPE tumor samples in the three labs. Measured total standard deviation including all sources of variation (i.e., tissue processing and RNA processing variability) was 2.9 ROR units, indicating that Prosigna™ measures a difference of 6.8 points between continuous ROR scores with 95% confidence. Concordance across the three labs for risk categorization in node-negative patients ranged from 88% (95% CI: 73-96) to 93% (95% CI: 80-98), and in node-positive patients, from 90% (95% CI: 77-96) to 95% (95% CI: 84-99).

In a 2014 study that supported FDA clearance of Prosigna™, Gnant et al. 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.[96] ABCSG-8 randomized HR-positive, postmenopausal women with early-stage breast cancer to five years of endocrine adjuvant therapy, either tamoxifen for five years or tamoxifen for two years followed by anastrozole for three years. Adjuvant or neoadjuvant chemotherapy was not allowed. Both PAM50 subtype and Prosigna™ ROR class were associated with 10-year distant recurrence-free survival, 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.
In 2014, Filipits et al. subsequently studied 919 patients who survived the first five years after treatment without recurrence.[98] Fifteen-year late-distant recurrence-free survival (i.e., years 5-15) was 98%, 90%, and 86% in ROR low-, intermediate-, and high-risk groups, respectively.

In 2013, Dowsett et al. 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.[99] Among 739 lymph node-negative patients, 10-year distant recurrence-free survival 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). Because proliferation-associated genes are given special weighting to produce the Prosigna™ ROR score, it is unclear how closely ROR46 approximated the marketed test; the authors reported a correlation of 0.9989 between ROR50, which incorporated all PAM50 genes, and ROR46 risk classifications.

In 2013, Sestak et al. reported on the prognostic ability of PAM50 ROR score in 940 (16%) of 5880 patients from the ATAC trial.[100] Thirty percent of patients were lymph node positive. Investigators modified the ROR scoring algorithm to exclude tumor size and defined cut points by the median for each outcome; patients were segregated into two rather than three risk classes. These modifications have not been validated and may increase considerably the risk of misclassification bias. Two outcomes were examined, distant recurrence during the first five years after completion of hormone therapy and after five years (up to 10 years). For the latter, the number of patients at risk at the start of the interval was not reported; in the first five years, 71 distant recurrences occurred. Finally, estimated uncertainty (e.g., variance) was not reported for either outcome. Although distant recurrence-free survival was longer in the low-risk than in the high-risk group, given the methodological flaws of the study, the meaning of these results is uncertain.

Nielsen et al. compared the PAM50 classifier with standard clinicopathologic factors as represented by Adjuvant! Online and with models based on immunohistochemistry for biomarkers of intrinsic subtypes.[101] The study used samples from patients diagnosed between 1986 and 1992 with ER-positive breast cancer, either higher-risk (e.g. with lymphovascular invasion) node-negative or node-positive disease, and treated with five 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 gene expression signature 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 sufficiently low-risk population wherein 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, this finding is likely not generalizable to other populations. In addition, the authors did not clearly identify a final model for clinical use.

The initial development of the PAM50 Breast Cancer Intrinsic Classifier was reported by Parker et al.[102] In an independent test set, the test using three categories of risk (low, intermediate, and high) was significantly prognostic (Log-rank p=0.0002).

Clinical Practice Guidelines
Currently NCCN does not address the use of the PAM50 Breast Cancer Intrinsic Classifier as an option when evaluating breast cancer patients for risk of recurrence.

Current ASCO\textsuperscript{[74]} guidelines on the use of biomarkers to guide decisions on adjuvant therapy for women with early-stage invasive breast cancer state that the PAM50 score is one of several tests that may be used in women with ER/PgR-positive, HER2-negative, node-negative breast cancer. The guideline authors consider this to be a strong recommendation based on high quality evidence. However, this recommendation is based on three studies. All three studies were industry sponsored prospective analyses on retrospectively collected cohorts, and focused on the clinical validity and the potential for the test to impact treatment decisions, but did not directly demonstrate clinical utility.

Conclusion

The majority of PAM50/Prosigna\textsuperscript{TM} studies suffered from confounding due to heterogeneous patient samples. It is therefore difficult to estimate outcomes for the patients of interest: ER\(^+\), HER2-negative, LN-negative patients not receiving chemotherapy. In addition, studies reporting 10-year outcomes have not consistently used the commercially available version of the test or used standardized cutpoints for risk category determination. This inconsistency limits the conclusions that can be drawn regarding the potential clinical utility of this test.

**BluePrint\textsuperscript{®} and TargetPrint\textsuperscript{®}**

**Description**

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.

BluePrint\textsuperscript{®} 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\textsuperscript{®}. BluePrint\textsuperscript{®} classifies breast cancer into basal type, luminal type or ERBB2-type. TargetPrint\textsuperscript{®} offers a quantitative assessment of ER, PR and HER2 overexpression in breast cancer. Both BluePrint\textsuperscript{®} and TargetPrint\textsuperscript{®} are intended for use with MammaPrint\textsuperscript{®}.

TargetPrint\textsuperscript{®} 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\textsuperscript{®} and BluePrint\textsuperscript{®}.

**Studies of BluePrint\textsuperscript{®} and TargetPrint\textsuperscript{®}**

In 2016, Wesseling et al. compared TargetPrint\textsuperscript{®} to IHC and in situ hybridization (ISH) testing for ER, PR, and HER2 in samples from 806 patients at 22 hospitals. The positive/negative agreement between IHC and TargetPrint\textsuperscript{®} was 96%/87% for ER, 84%/74% for PR, and 74%/98% for HER2.\textsuperscript{[103]} The authors noted substantial discord in IHC/ISH results between different hospitals and indicated that TargetPrint\textsuperscript{®} might improve the reliability of these discordant results by prompting retesting in a
In 2015, Gran et al. compared HER2 testing results by IHC, FISH, and TargetPrint® in 127 tumor specimens from patients with early-stage breast cancer in South Africa. Tumor specimens were fresh frozen (32%) or FFPE (68%). Only specimens with IHC-positive results (n=23) underwent FISH testing, except for one IHC-negative specimen that had a positive TargetPrint® result, subsequently confirmed by reflex FISH. TargetPrint® improved HER2 testing compared with IHC/FISH in four (17%) of 24 cases that underwent both IHC and FISH testing. TargetPrint® performance in this study cannot be fully characterized in the absence of FISH testing of IHC-negative samples.

In 2014, Whitworth et al. reported reclassification of 94 (22%) of 426 patients with breast cancer who were classified by both IHC/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.

In 2014, Viale et al. reported concordance between TargetPrint® and IHC testing for ER and 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 agreement were 83% and 91%, respectively; PPV and NPV were 97% and 59%, respectively. For HER2, positive and negative percent agreement were 75% and 99%, respectively; PPV and NPV were 91% and 97%, respectively.

Nguyen and colleagues undertook a comparison of molecular subtyping with BluePrint®, MammaPrint® and TargetPrint® to locally assess clinical subtyping using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). The three 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 were performed on 132 samples. The concordance between BluePrint® and IHC and FISH testing was 94% for both the basal-type and luminal-type subgroups, and 95% for the HER2-type. The concordance of BluePrint® with subtyping using mRNA readout (TargetPrint®) was 98% for the basal-type, 96% for the luminal-type, and 97% for the HER2-type. The authors concluded that implementation of these multigene assays may improve the clinical management of breast cancer patients by including substratification rather than tumor grade alone.

The BluePrint® molecular subtyping profile was developed using 200 breast cancer specimens that had concordant ER, PR and HER2 protein levels by immunohistochemistry and TargetPrint® mRNA readout. Using a threefold cross validation procedure, the 80 genes thought to best discriminate the three molecular subtypes were identified. BluePrint® was confirmed on four independent validation cohorts (n=784), which included patients from a consecutive series of patients seen at Netherlands Cancer Institute and treated with adjuvant tamoxifen monotherapy (n=274), a group of patients from the RASTER trial (n=100), and two publicly available data sets (n=410). In addition, 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 pathologic complete response (pCR) among molecular subgroups compared with local pathology: 56% of the 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.

Clinical Practice Guidelines

Currently, neither NCCN nor ASCO recommend BluePrint® and TargetPrint® as an option when evaluating breast cancer patients for risk of recurrence. However, in 2010, ASCO and the College of American Pathologists (CAP) issued recommendations on immunohistochemical testing for ER and PR, and issued recommendations in 2007\[41,109\] (updated in 2014)\[110\] for HER2 testing by immunohistochemical and FISH methods. Recommendations do not address the use of gene expression assays to test for ER, PR or HER2 expression. These tests were not addressed in the ASCO 2016 guidelines for biomarkers.

BreastPRS™

Description

BreastPRS™ is a gene expression assay that analyzes 200 genes in its algorithm, and was validated from a meta-analysis of publically available genomic datasets.\[111\] BreastPRS™ is a binary assay which stratifies patients into low- and high-risk groups.\[112\]

Studies of BreastPRS™

D’Alfonso et al. sought to translate a previously published validation study of BreastPRS™, using fresh-frozen tissue, to formalin-fixed paraffin-embedded (FFPE) tumor samples.\[112\] The authors compared the BreastPRS prognostic index to the Oncotype DX® assay and correlated recurrence scores with clinicopathologic features. They also used publically available whole genome profiles from a series of untreated ER+ node negative patients to investigate the ability of BreastPRS™ to reclassify Oncotype DX® intermediate-risk patients into high- versus low-risk categories with clinically significant differences in outcome. A linear relationship of the BreastPRS™ prognostic score was observed between fresh-frozen and FFPE formats. BreastPRS™ recurrence scores were compared with Oncotype DX® recurrence scores from 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 the 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<0.0001). Fifty-nine of 260 (23%) patients from four 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 (p=0.029, HR: 3.64, 95% CI: 1.40-9.50). At 10 years from diagnosis, the low-risk group had a 90% recurrence-free survival (RFS) rate compared to 60% for the high-risk group. The authors concluded that the BreastPRS™ recurrence score is comparable with Oncotype DX® and can reclassify Oncotype DX® intermediate-risk patients into two groups with significant differences in RFS. The authors noted further studies are necessary to validate these findings.

Clinical Practice Guidelines
Currently, neither NCCN nor ASCO address the use of BreastPRS™ as an option when evaluating breast cancer patients for risk of recurrence.

**EndoPredict®**

**Description**

EndoPredict® is a gene expression test that uses reverse transcription polymerase chain reaction (RT-PCR) of 12 genes.

**Studies of EndoPredict®**

In 2011, Filipits et al. reported on the validation of Endopredict® using tumor samples from women receiving endocrine treatment in the ABCSG-6 and ABCSG-8 trials.[113] The test was successful in 378 out of 395 tumors from ABCSG-6 and 1,324 out of 1,330 tumors from ABCSG-8. All tumors were HER2-negative. Prespecified cutoff points were used to classify the patients into EP and EPclin high and low risk groups (5 for EP, 3.3 for EPclin). The EPclin score combines the EP risk score with two clinical parameters, tumor size and nodal status. The 10-year distant recurrence rates for the EP low and high risk groups from ABCSG-6 were 8% (95% CI: 3% to 13%) and 22% (95% CI: 15% to 29%), respectively, and the rates for the EP low and high risk groups from ABCSG-8 were 6% (95% CI: 2% to 9%) and 15% (95% CI: 11% to 20%), respectively. The EPclin score outperformed the EP score in this study, with 10-year distant recurrent rates of 4% (95% CI: 1% to 8%) and 28% (95% CI: 20% to 36%) in the ABCSG-6 low and high risk groups, respectively, and 4% (95% CI: 2% to 5%) and 22% (95% CI: 15% to 29%) in the ABCSG-8 low and high risk groups.

A 2016 study by Buus et al. evaluated Endopredict® as a prognostic indicator for breast cancer recurrence in women treated endocrine therapy.[114] This study was performed with 928 ER-positive, HER2-negative tumors samples from the TransATAC trial, which randomized post-menopausal women with localized disease to either tamoxifen or anastrozole for five years. High and low risk groups for both EP and EPclin were determined using pre-specified cutpoints. The 10-year recurrence rate for node-negative patients was 3.0% (95% CI: 1.5-6.0) for the EP low group and 14.5% (95% CI: 11.3-18.8) for the EP high group. For the node-negative EPclin low and high groups, the 10-year recurrence rates were 5.9% (95% CI: 4.0-8.6) and 20.0% (95% CI: 14.6-27.0), respectively. The 10-year recurrence rates were also determined for node-positive patients: 21.3% (95% CI: 13.9-31.9) for the EP low group, 36.4% (95% CI: 29.6-40.1) for the EP high group, 5.0% (95% CI: 1.2-18.) for the EPclin low group, and 36.9% (95% CI: 30.2-44.5) for the EPclin high group.

Bertucci et al. evaluated 553 ER+/HER2-negative breast cancers treated with anthracycline-based neoadjuvant chemotherapy.[115] 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 five-year disease-free survival was 88% (95% CI: 81-95) in the EndoPredict® low-risk group and 73% (95% CI: 63-85) in the EndoPredict® high-risk group.

Martin et al. assessed tumor samples from 566 ER-positive, HER2-negative patients who participated in the GEICAM 9906 RCT.[116] GEICAM 9906 compared two 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 eight weekly courses of paclitaxel (FEC-P). EP was successfully assayed in 555 (98%) of 566 tumor samples. There were 25% (n=141) of
the samples 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: 2.5-9.6; 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. examined predictive ability of EP and EPclin for early (0-5 years) and late (>5 years post-diagnosis) disease recurrence.[117] Tumor samples from chemotherapy-untreated, ER-positive, HER2-negative patients who participated in one of two RCTs (ABCSG6 or ABCSG8) were assayed (total N=1702). In the trials, patients received either tamoxifen for five years or tamoxifen for two years followed by anastrozole for three 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 (i.e., HRs) of distant recurrence were reported. In comparison with low-risk patients, high-risk patients had an almost three-fold increase in the risk of recurrence in the first five years after diagnosis (HR: 2.80; 95% CI: 1.81-4.34; log-rank test, p<0.001) and a slightly increased risk after five years in those who survived five years (HR: 3.28; 95% CI: 1.48-7.24; 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 five-fold risk of recurrence within the first five years (HR: 4.82; 95% CI: 3.12-7.44; log-rank test, p=0.001) and a more than six-fold increased risk of recurrence after five years (HR: 6.26; 95% CI: 2.72-14.36; log-rank test, p<0.001).

Additional Applications of EndoPredict®

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.[118] 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 six 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.

Clinical Practice Guidelines

Currently, NCCN[111] does not address the use of the EndoPredict® test.

Current ASCO[74] guidelines on the use of biomarkers to guide decisions on adjuvant therapy for women with early-stage invasive breast cancer state that the EndoPredict® is one of several tests that may be used in women with ER/PgR-positive, HER2-negative, node-negative breast cancer. The strength of this recommendation is moderate, indicating that the guideline authors have moderate confidence that the recommendation reflects best practice, and it is judged to be based on intermediate quality evidence.

Test Comparison Studies

Sgroi et al. compared the Breast Cancer IndexSM and Oncotype DX® in 665 lymph node-negative women receiving endocrine therapy but not chemotherapy in the ATAC trial.[76] 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 two 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. compared the risk of recurrence (ROR) score generated by PAM50 to the Oncotype DX® 21-gene recurrence score (RS), four immunohistochemical markers (IHC4) for ER, PR, Ki67 and HER2, and a clinical treatment score (CTS).[99] Patients had ER-positive, primary breast disease treated with anastrozole or tamoxifen in the ATAC trial, a double-blinded, phase three clinical trial that was designed to compare the ability of anastrozole, tamoxifen, and the two drugs in combination to prevent breast cancer recurrence in postmenopausal women with hormone receptor-positive tumors. Lymph node-negative and positive patients were included. mRNA from 1,017 patients was assessed for ROR, and likelihood ratio (LR) 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<.001), and in all four 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 performed a systematic review of the literature on the clinical validity/utility, change in clinical practice, and economic implications of early-stage breast cancer stratifiers.[119] There were 56 articles that 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). The results of the review found that Oncotype DX® recurrence score satisfied level I evidence for estimating distant recurrence risk (DRR), OS, and response to adjuvant chemotherapy, and level II evidence for estimating local recurrence risk. MammaPrint® and MammaPrint® satisfied level II evidence for estimating DRR and OS. Adjuvant! Online satisfied level II evidence for estimating DRR, OS, and chemotherapy response. BreastOncPx™ satisfied level III evidence for predicting DRR and OS. Ten studies reported changes in clinical practice patterns using the 21-gene recurrence score. Overall, the 21-gene recurrence score was associated with change in treatment recommendations and/or decisions in 20.6-74.0% of cases.

Varga et al. analyzed the EndoPredict® (EP) test in 34 hormone positive, invasive breast cancer cases and compared the EP scores with the Oncotype DX® Recurrence-scores (RS) obtained from the same cancer samples.[120] EP classified 11 patients as low-risk and 23 patients as high-risk, whereas the RS Score defined 15 patients as low-risk, 10 patients as intermediate-risk in and nine patients as high-risk. There were major discrepancies in six of 34 cases (18%), with low-risk RS classified as high-risk by EP in six 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.

Similarly, the study by Buus et al., described earlier, compared EndoPredict® (EP) with Oncotype DX® RS in hormone receptor-positive, HER2-negative tumor samples from the TransATAC study.[114] The EP assay was used to generate an EPclin value that incorporated information about nodal status and tumor size. In this study, EP, EPclin, and RS had similar predictive power for distant recurrence in 0-5 years in node-negative disease, while EP and EPclin had more prognostic value than RS for distant
recurrence in 5-10 years, regardless of nodal status. Classification as low-risk by EPClin was associated with significantly lower 10-year risk of recurrence than a low-risk classification by RS (EPClin: 5.8%, 95% CI = 4.0 to 8.3; RS: 10.1%, 95% CI = 7.7 to 13.1). EPClin classification as high-risk was also more highly associated with cases of recurrence than non-low-risk RS classification. However, for this analysis, both intermediate risk and high risk RS categories were grouped together to allow comparison between the two risk categories of EPClin and the three risk categories of the RS.

Fan et al. used five 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 one with one 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 Oncotype DX® Recurrence Score, the 70-gene signature (MammaPrint®), and the H/I Ratio in 153 patients with ER-positive breast cancer treated with adjuvant tamoxifen. Of these patients, 38% 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 H/I 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 two 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).

Summary

Oncotype DX®

Oncotype DX® Assay in Node-Negative Patients

There is enough research to show that the Oncotype DX® test can help identify patients with certain types of breast cancer that may be at low risk for disease recurrence, and can be useful when making decisions about chemotherapy treatment. Clinical guidelines based on research consider this test to be an option to help in making treatment decisions for women with breast cancer who do not have lymph node involvement. Therefore, Oncotype DX® testing may be considered medically necessary in lymph node-
negative patients when policy criteria are met.

**Oncotype DX® Assay in Node-Positive Patients**

There is enough research to show that the use of the Oncotype DX® test may not improve health outcomes in node-positive breast cancer patients. For women with node-positive breast cancer, the risk of cancer recurrence without additional recommended therapy may be too high. Therefore, Oncotype DX® testing in node-positive patients is considered not medically necessary.

**Oncotype DX® Assay in DCIS Patients**

There is not enough research to show that using Oncotype DX® DCIS helps women with ductal carcinoma in situ (DCIS) make treatment decisions that improve health outcomes. Therefore Oncotype DX® DCIS is considered investigational.

**Oncotype DX® Assay in Men**

There is not enough research on using Oncotype DX® to help make treatment decisions for men with primary breast cancer. Therefore, use of the Oncotype DX® assay in men is considered investigational.

**Oncotype DX® Assay to Determine or Confirm HER2 Status**

Guidelines based on research recommend using other methods and not Oncotype DX® to confirm HER2 status. Therefore, use of the Oncotype DX® assay to determine or confirm HER2 status is considered investigational.

**MammaPrint®**

There is not enough research to show that using the MammaPrint® (70-gene signature) test to make treatment decisions can improve health outcomes for breast cancer patients. There are no clinical guidelines based on research that recommend using MammaPrint® to make treatment decisions for women with any type of early breast cancer. Therefore, MammaPrint® testing is considered investigational.

**Breast Cancer IndexSM**

It appears that the Breast Cancer IndexSM test can help identify patients with certain types of breast cancer that may be at low risk for disease recurrence, and can be useful when making decisions about chemotherapy treatment. Clinical guidelines based on research consider this test to be an option to help women with breast cancer make treatment decisions. Therefore, Breast Cancer IndexSM testing may be considered medically necessary in lymph node-negative patients when policy criteria are met.

**EndoPredict®**

It appears that the EndoPredict® test can help identify patients with certain types of breast cancer that may be at low risk for disease recurrence, and can be useful when making decisions about chemotherapy treatment. Clinical guidelines based on research consider this test to be an option to help women with breast cancer make treatment decisions. Therefore, EndoPredict® testing may be considered medically necessary in lymph node-negative patients when policy criteria are met.
Molecular Grade Index (Aviara MGI\textsuperscript{SM}), Mammostrat\textsuperscript{R}, BreastOncPx\textsuperscript{TM}, Prosigna \textsuperscript{TM}, NexCourse\textsuperscript{R} Breast IHC4, BreastPRS\textsuperscript{TM}

There is not enough research to show that the Molecular Grade Index (Aviara MGI\textsuperscript{SM}), Mammostrat\textsuperscript{R}, BreastOncPx\textsuperscript{TM}, Prosigna \textsuperscript{TM}, NexCourse\textsuperscript{R} Breast, or BreastPRS\textsuperscript{TM} tests can help breast cancer patients make treatment decisions that improve health outcomes. Therefore, the Molecular Grade Index (Aviara MGI\textsuperscript{SM}), Mammostrat\textsuperscript{R}, BreastOncPx\textsuperscript{TM}, PAM50 Breast Cancer Intrinsic Classifier\textsuperscript{TM}, NexCourse\textsuperscript{R} Breast, and BreastPRS\textsuperscript{TM} tests are considered investigational.

BluePrint\textsuperscript{R} and TargetPrint\textsuperscript{R}

There is not enough research to show that BluePrint\textsuperscript{R} and TargetPrint\textsuperscript{R} improve health outcomes in women with breast cancer. There are no clinical guidelines based on research that recommend using BluePrint\textsuperscript{R} or TargetPrint\textsuperscript{R} to help determine the risk of cancer recurrence for breast cancer patients. Therefore, the gene expression assays BluePrint\textsuperscript{R} and TargetPrint\textsuperscript{R} are considered investigational for all indications.

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**CROSS REFERENCES**

[Genetic Testing for Hereditary Breast and/or Ovarian Cancer](#), Genetic Testing, Policy No. 02

[Genetic and Molecular Diagnostic Testing](#), Genetic Testing, Policy No. 20

[Gene Expression Profiling for Melanoma](#), Genetic Testing, Policy No. 29

[Evaluating the Utility of Genetic Panels](#), Genetic Testing, Policy No. 64

[Detection of Circulating Tumor Cells in the Management of Patients with Cancer](#), Laboratory, Policy No. 46
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