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Medical Policy Manual

Topic: Assays of Genetic Expression in Tumor Tissue as a Technique to Determine Prognosis In Patients With Breast Cancer

Date of Origin: October 5, 2004

Section: Genetic Testing

Last Reviewed Date: March 2014

Policy No: 42

Effective Date: June 1, 2014

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

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 (ER) positive, and lymph node negative. These women have an approximately 15% baseline risk of recurrence; approximately 85% of these patients would be disease-free at ten 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 chemotherapy than can benefit. Better predictors of baseline risk could help women who prefer to avoid chemotherapy if assured that their risk is low, make better treatment decisions in consultation with their physicians.

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)
- 70-gene signature MammaPrint® (also referred to as the "Amsterdam signature"; Agendia)
- Mammostrat™ (Clarient Diagnostic Services)
- Molecular Grade Index (Aviara MGI^{SM}; AviaraDx, Inc.)
- Breast Cancer Index^{SM}, a combination of the Molecular Grade Index (MGI) and the HOXB13:IL17BR Index (bioTheranostics)
- BreastOncPx^{TM} (Breast Cancer Prognosis Gene Expression Assay; LabCorp)
- PAM50 Breast Cancer Intrinsic Classifier^{TM} (ARUP National Reference Laboratory)
- NexCourse® Breast IHC4 (Geneoptix)
- BreastPRS™ (Signal Genetics)
- EndoPredict™ (Sividon Diagnostics)
- BluePrint® (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, using a slightly different algorithm to calculate results, is also 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.

Note: This policy does not address the identification of germ-line 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 the plan's Medical Policy Genetic Testing for Hereditary Breast and/or Ovarian Cancer, Genetic Testing No. 02.

MEDICAL POLICY CRITERIA

I. The use of Oncotype DX® in women with primary breast cancer to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy may be considered **medically necessary** when all of the following characteristics are present:

A. Primary tumor size 0.6-1cm with moderate/poor differentiation or unfavorable features, OR tumor size > 1 cm.

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.

B. Hormone receptor positive (that is ER-positive or PR-positive)

C. HER2-negative

D. Negative lymph nodes (nodes with micrometastases less than 2 mm in size are considered node negative)

E. The patient will be treated with adjuvant endocrine therapy, e.g., tamoxifen or aromatase inhibitors; AND

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® 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® 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® are considered **investigational**, including but not limited to:

A. Predicting response to specific chemotherapy regimens

B. Determining HER2 status

V. Use of a subset of genes from the 21-gene RT-PCR assay (i.e., Oncotype DX DCIS) for predicting recurrence risk in patients with noninvasive ductal carcinoma in situ to inform treatment planning following excisional surgery is considered **investigational**.

VI. Use of a subset of genes from the 21-gene RT-PCR assay (i.e., Oncotype DX DCIS) in men with primary breast cancer to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy is considered **investigational**.

VII. 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. Breast Cancer Index[SM]

E. BreastOncPx[TM]

F. PAM50 Breast Cancer Intrinsic Classifier[TM]

G. NexCourse® Breast IHC4 (Geneoptix)

H. BreastPRS™

I. EndoPredict™

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.**

SCIENTIFIC EVIDENCE[1]

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 in order to reliably establish the clinical utility of the assay in question.

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 indications for the test are newly diagnosed breast cancer patients with stage I or II disease that is node-negative and ER-positive, and who will be treated with tamoxifen.

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 already-completed randomized controlled trials 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 1 study, multifocal tumors were excluded.[2]

Oncotype DX® in Lymph Node-Negative Patients

Validation Studies in Lymph Node-Negative Patients

A few studies attempted to delineate the association between RS and recurrence risk in node-negative patients.[3-6] Results indicate strong, independent associations between Oncotype DX® RS results and distant disease recurrence or death from breast cancer.[5,7]

- In secondary analyses of the Paik et al. 2004a data,[4,5] patient risk levels were individually classified by conventional risk classifiers, then re-classified by Oncotype DX®. Oncotype DX® adds additional risk information to the conventional clinical classification of individual high-risk patients, and identifies a subset of patients who would otherwise be recommended for chemotherapy but are actually at lower risk of recurrence (average 7%-9% risk at 10 years; upper 95% confidence interval [CI] limits, 11%-15%). Thus, a woman who prefers to avoid the toxicity and inconvenience of chemotherapy and whose Oncotype DX® RS value shows that she is at very low risk of recurrence might reasonably decline chemotherapy. The lower the RS value, the greater the confidence that the woman can have that chemotherapy will not provide net benefit; outcomes are improved by avoiding chemotherapy toxicity.

- An additional study, in which samples from a randomized controlled trial of ER-positive, node-negative breast cancer patients treated with tamoxifen versus tamoxifen plus chemotherapy were tested by Oncotype DX®, provides supportive evidence.[2] RS high-risk patients derived clear benefit from chemotherapy whereas the average benefit for other patients was statistically not significant, although the confidence intervals were wide and included the possibility of a small benefit.

Technology Assessments

The BlueCross BlueShield Association (BCBSA) Technology Evaluation Center (TEC) published two technology assessments on gene expression profiling, one in 2005, followed by an update in 2008.

- The 2005 TEC Assessment concluded that due to the lack of published evidence supporting clinical utility, gene expression profiling for managing breast cancer treatment did not meet the technology assessment criteria.[7]
- The 2008 TEC Assessment on gene expression profiling of breast cancer to select women for adjuvant chemotherapy concluded that Oncotype DX® meets criteria for women with characteristics similar to those in the validation studies.[8] Patients in the validation studies were less than 70 years of age (or had a life expectancy of 10 years or more), had unilateral, non-fixed, estrogen-receptor (ER) positive, node-negative (by full axillary dissection) carcinomas and were treated with surgery (mastectomy or lumpectomy), radiation therapy, and tamoxifen. In one trial, patients in the experimental arm were also treated with cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) chemotherapy. Most (92%) patients were negative for HER-2.[3]

  Because clinical care for breast cancer patients has evolved since the original trials from which archived samples were acquired for assay validation, differences in evaluation and treatment regimens were considered. It was concluded that 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[9], and therefore may not be prescribed in this population.

- Lymph nodes with micrometastases are not considered positive for purposes of treatment recommendations.[10] Current practice largely involves a detailed histologic examination of sentinel lymph nodes allowing for the detection of micrometastases (less than 2 mm in size).

- Those whose tumors are 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.[11,12]

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

Subsequent to the publication of the 2008 TEC Assessment, several nonrandomized studies reporting on the use of the 21-gene assay in lymph-node positive patients have been published:

- 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 3 RS categories as in the original validation studies.[13]
- 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.[14]
- Tzeng examined how women receive and incorporate the results of their 21-gene profiles using mailed survey and chart review.[15] About two-thirds of women believed they understood most or all of what they were told about their recurrence risk based on their test results; the majority who experienced test-related distress had intermediate or high estimated recurrence risks by RS result. The objective, recalled, and perceived recurrence risks by women in the study were surprisingly similar, and 95% agreed that the test gave them a better understanding of their treatment options and chances of success. However, about one-third of women believed they understood only a moderate amount or less during these discussions. The study was limited in generalizability in that participants were mostly Caucasian, well-educated women who had health insurance and came from urban areas.
- Tang et al. compared the prognostic and predictive utility of RS and Adjuvant! in the NSABP B-14 and B-20 trial patients.[16] 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.[17-23] In general, these studies report that comparing recommendations made prior to and revised after knowledge of RS results show that decisions change in about 30-40% of patients, most often from endocrine therapy plus chemotherapy to endocrine therapy alone. For example, in a retrospective reclassification analysis, Joh et al. found that inclusion of the Oncotype DX recurrence scores resulted in a 24.9% change in (after the fact) treatment recommendations, resulting in fewer patients projected to receive chemotherapy.[22] Hassett et al. evaluated registry data from the National Comprehensive Cancer Network (NCCN) Breast Cancer Outcomes Database Project focusing on women diagnosed for hormone-receptor (HR)-positive stage I to III unilateral breast cancer during 2006-2008. Compared to women with Oncotype DX-determined intermediate-risk cancer, women with Oncotype-determined high-risk cancers were more likely to receive chemotherapy (odds ratio [OR]: 12.0; 95% CI: 6.7 to 21.3) and women with low-risk cancers were less likely to receive chemotherapy (OR: 0.1; 95% CI: 0.1 to 0.2)[23]. Some view these as evidence of clinical utility because more patients avoid the toxicity of chemotherapy,[24] however there are no patient outcomes attached to these studies; 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. For example, Lo et al. conducted a prospective multicenter study that examined both physician and patient treatment selection, as well as the impact of the RS result on patients' anxiety, quality of life, and satisfaction with choice of treatment. However, 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.[17]

<u>Oncotype DX® in Lymph Node-Positive Patients</u>

Technology Assessments

In 2010, a BCBSATEC assessment addressed the use of the 21-gene expression assay (Oncotype DX®) in lymph node-positive breast cancer patients.[25] The following is a summary of their findings:

The major publication reviewed is a report from the Southwest Oncology Group (SWOG) trial. The trial arms included a postsurgery group treated only with tamoxifen and a comparison group in which patients were treated with chemotherapy followed by tamoxifen. The effect of the 21-gene RS on disease-free survival (DFS) and overall survival (OS) was evaluated by treatment group in order to examine whether it identified those who might benefit from anthracycline-based chemotherapy. SWOG findings suggested that the 21-gene expression profile RS was prognostic in patients treated only with tamoxifen; however, it is unknown whether the RS improved risk classification compared with standard clinical and histopathologic characteristics. The study results also suggested that among women with lymph-node-positive, ER-positive breast cancer, the 21-gene expression profile (Oncotype DX®) may help identify those who are unlikely to benefit from the addition of CAF chemotherapy to their treatment regimen, despite the higher risk of recurrence positive nodes. However, the event rate was low, especially in the low-risk RS group, resulting in broad confidence intervals that included the possibility of benefit from chemotherapy. Overall, due to the lack of clear and sufficient information, there is a need for a second, confirmatory study.

The assessment also noted that additional evidence in the form of single-arm trials suggested that patients with lymph-node-positive breast cancer who are treated with chemotherapy are more likely to respond if their RS is high than if it is low, but control arms for comparison were lacking. Lack of

adequate comparison group limits ability to control for the many types of bias that can affect study outcomes.

The TEC Assessment concluded that use of the 21-gene expression profile for selecting adjuvant chemotherapy in patients with lymph-node-positive breast cancer did not meet the TEC criteria.

Studies of Oncotype DX® in Lymph Node-Positive Patients

Recently, there has been an interest in understanding the association between RS and recurrence risk in node-positive patients. However, the results from these studies have not been able to establish strong, independent associations between Oncotype DX® RS and distant disease recurrence or death from breast cancer in node-positive patients:

- 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.[26] 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.
- 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.[27] They evaluated 8814 samples from the Southwest Oncology Group Trial, in which outcomes from randomized node-positive, ER-positive patients treated with tamoxifen for 5 years were compared with those treated with cyclophosphamide, doxorubicin, fluorouracil (CAF) chemotherapy followed by tamoxifen (CAF-T) for 5 years. Samples were available for determination of RS for 41% (n=148) and 39% (n=219) of the trial arms, respectively. In this study, 10-year disease-free survival (DFS) and overall survival (OS) outcomes in the tamoxifen study arm differed by RS risk category (p=0.017 and 0.003, respectively), indicating that the RS is prognostic. When the 2 treatment arms were compared within RS risk categories, only patients in the high RS category significantly benefited from the addition of CAF to tamoxifen (for DFS, 42% [tamoxifen] vs. 55% [CAF-T], p=0.033; for OS, 51% [tamoxifen] vs. 68% [CAF-T], p=0.027), suggesting that RS was also predictive of response to chemotherapy. A multivariable analysis of RS interaction with DFS, adjusted for number of positive nodes, was significant for the first 5 years of follow-up at p=0.029, and remained significant after adjusting for age, race, tumor size, progesterone receptor status, grade, p53, and HER2. However, the interaction was not significant (p=0.15) after adjusting for ER level (ER gene expression is a component of the 21-gene profile). Interaction results were similar for OS. 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.
- 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.[28] 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 1-3 involved nodes, and 63 patients 4 or more; these were not evaluated separately. Rates of distant recurrence at 9 years were 17% (95% CI, 12-24%), 28% (20-39%), and 49% (35-64%), respectively. It is not clear that the risk of distant recurrence in low-risk RS patients would be sufficiently low to forgo the choice of chemotherapy. The authors note that their study "did not directly evaluate the value of RS in predicting the benefit of chemotherapy." In addition, the HER2 status was not taken into consideration in this retrospective analysis.

- 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.[29] 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.
- 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.[30] 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.[22] 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.

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.[31] 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. Similarly, guidelines for HER2 testing specify IHC and/or fluorescence in situ hybridization (FISH) methods.[32] Although the HER2 component of the 21-gene assay has been shown to strongly correlate with FISH results,[33] the 21-gene assay should not be ordered to determine or confirm HER2.

Clinical Practice Guidelines

- The 2014 National Comprehensive Cancer Network (NCCN) guidelines consider Oncotype DX® assay as "an option when evaluating patients with primary tumors characterized as 0.6-1.0 cm with unfavorable features of >1 cm, and node-negative, hormone receptor-positive and HER2-negative (evidence category 2A: the recommendation is based on lower level evidence and there is uniform NCCN consensus). In this circumstance, the recurrence score may be determined to assist in estimating likelihood of recurrence and benefit from chemotherapy (evidence category 2B: the recommendation is based on lower level evidence and there is nonuniform NCCN consensus, but no major disagreement). The panel emphasizes that the recurrence score should be used for decision-making only in the context of other elements of risk stratification for an individual patient. All recommendations involving use of the recurrence score in the treatment decision-making are categorized as 2B."[34] Per the guideline, patient selection for assay use in lymph node-positive patients remains controversial (evidence category 3: based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate).

- The American Society of Clinical Oncology (ASCO) "2007 Update of Recommendations for the use of Tumor Markers in Breast Cancer" indicates that the Oncotype DX® assay can be used to predict the risk of recurrence in newly diagnosed patients with node-negative, estrogen-receptor positive breast cancer, who will be treated with tamoxifen; and to identify patients who may not require adjuvant chemotherapy. The recommendations state, "Although performed retrospectively, the validation of this assay using a prospectively collected clinical trial data set, but retrospectively collected tissues from the data set, might be considered as Level of Evidence I [best] for use of this assay."[35]

Patients with 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 5 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.[36]

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).[37] DCIS Score was significantly associated with recurrence outcomes (HR: 2.34 per 50 units; 95% CI: 1.15, 4.59; p=0.02) whether or not patients were treated with tamoxifen. The standard Oncotype DX Score for early breast cancer was not associated with DCIS recurrence outcomes.

<u>Conclusion</u>

These studies address the development of the Oncotype DX DCIS Score and the clinical validity (association of the test result with recurrence outcomes). Evidence for the clinical utility of Oncotype DX DCIS is limited; whether women are better categorized as to their recurrence risk by the Oncotype DX DCIS Score compared with standard clinical indicators of risk has not yet been addressed.

Oncotype DX® in Men

Researchers have focused relatively little attention on male breast cancer compared with female breast cancer.[38] 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 factor 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. Five percent to 10% of men with BRCA2 mutations (and a smaller proportion of those with BRCA1 mutations) eventually develop breast cancer.

Evidence of OncotypeDx utilization in men is limited to a single case study[39] and one non-randomized study[18] that includes 1 male patient out of 29 patients. No conclusions can be made on a study that included one male patient. 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.[38] 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.

MammaPrint®

Description

MammaPrint® has received 510(k) clearance for marketing by the FDA; it is 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.

Technology Assessments

The BlueCross BlueShield Association (BCBSA) Technology Evaluation Center (TEC) published two technology assessments on gene expression profiling, one in 2005, followed by an update in 2008.

- The 2005 TEC Assessment concluded that due to the lack of published evidence supporting clinical utility, gene expression profiling for managing breast cancer treatment did not meet the technology assessment criteria.[7]

- The 2008 TEC Assessment[8] reviewed the available studies[40-44] and found insufficient evidence to determine whether MammaPrint® is better than conventional risk assessment tools in predicting recurrence. Limited technical performance evaluation of the commercial version of the assay suggests good reproducibility. Recurrence rates of patients classified as low risk in available studies were 15%-25%, likely too high for most patients and physicians to consider forgoing chemotherapy. Similarly, in one study, after Adjuvant! risk classification, patients reclassified as low risk by the 70-gene signature in either Adjuvant! risk group had 10-year disease-free survival rates of 88%–89%, with lower confidence limits of 74%-77%. Patients reclassified as high risk had 10-year disease-free survival rates of 69%, with lower confidence limits of 45%–61% and upper confidence limits of 76%–84%; ROC analysis suggests only a small improvement with MammaPrint® classification compared with a conventional classifier.[40]

Other Studies of MammaPrint®

- 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.[45] 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). The 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 5 years. Since most clinical relapses in lymph node negative, ER positive breast cancers do not occur until 5 or even 10 years after diagnosis, with or without the use of adjuvant therapy, the study data should be considered not yet mature.
- Because initial studies had been conducted on samples from younger patients (age less than 61), 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.[46] 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.[47] However, the small study size and small number of events precludes an adequate statistical analysis.[48] 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 an ROC analysis also showed overlapping confidence intervals.[40]

- Mook et al studied 241 node-positive patients with primarily ER-positive, HER2-negative tumors treated variably.[49] 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 reclassification 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,[15] reporting 98% (95% CI, 94-100%) 10-year breast cancer-specific survival for good prognosis signatures vs. 64% (52-76%) for poor prognosis signatures; adjusted HR 3.63 (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.[50]
- The Microarray Prognostics in Breast Cancer (RASTER) study was designed to assess feasibility of implementation and impact on treatment decisions of the MammaPrint 70-gene signature, as well as recurrence outcomes. Five-year follow-up results were presented at the 8th European Breast Cancer Conference.[51] (46) The study followed 427 node-negative, early-stage breast cancer patients who participated in the RASTER Study and had a 70-gene signature (MammaPrint), which was available to help direct post-surgery treatment decisions, and which was compared to Adjuvant! Online. In the group that did not receive any adjuvant therapy, the patients classified as low risk by both the 70-gene signature and by Adjuvant! Online (n=88) had a distant disease-free survival (DDFS) of 95% (95% CI: 90-100%), and patients originally classified high risk by Adjuvant! Online but low risk by the 70-gene signature (n=70) had a similarly high DDFS of 100%. The results suggest that MammaPrint is a better prognostic classifier than standard clinical and pathological classifiers. However, the patient numbers are low, and event numbers very low, making firm conclusions difficult. The results are also for 5-year recurrence, although because the study is not yet mature, 4-year recurrence rates would be more representative.
- 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.[52] 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.
- 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.[48]
- A few other studies of MammaPrint® have been published, however the studies continue to be small and/or retrospective or pooled re-analyses of subgroups from previously published retrospective studies.[53-59]

Clinical Practice Guidelines

Currently, neither NCCN nor ASCO recommend MammaPrint® as an option when evaluating breast cancer patients for risk of recurrence.

THEROS Breast Cancer Index℠

Description

The Breast Cancer Index[SM] is a simultaneous assessment of THEROS H/ISM (HOXB13:IL17BR, formerly Aviara H/ISM) and THEROS MGISM (Molecular Grade Index, formerly Aviara MGISM).

Technology Assessments

The BlueCross BlueShield Association (BCBSA) Technology Evaluation Center (TEC) published two technology assessments on gene expression profiling, one in 2005, followed by an update in 2008.

- The 2005 TEC Assessment concluded that due to the lack of published evidence supporting clinical utility, gene expression profiling for managing breast cancer treatment did not meet the technology assessment criteria.[7]
- The 2008 TEC Assessment[8] reviewed the available studies[48,60-64] and found insufficient evidence to determine whether the Breast Cancer Index[SM] is better than conventional risk assessment tools in predicting recurrence. Assay configuration and performance characteristics of the commercially available version of the test have not been published. Recurrence rates of patients classified as low risk in available studies were 17%-25%, likely too high for most patients and physicians to consider forgoing chemotherapy. There are no reclassification studies to directly compare the THEROS Breast Cancer Index[SM] with conventional risk classifiers.

Other Studies of Breast Cancer Index[SM]

- 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.[65] The continuous model was also categorized resulting in proportions of low, intermediate, and high-risk patients similar to those reported in the Ma et al. study.[66] This continuous predictor was tested in patients from the no adjuvant treatment arm (n=274) of the same clinical trial, with estimates of rates of distant metastasis at 10 years in the low, intermediate, and high risk groups of 8.3% (95% CI: 4.7–14.4), 22.9% (14.5–35.2) and 28.5% (17.9–43.6), respectively. The estimates of breast cancer-specific death were 5.1% (95% CI: 1.3–8.7), 19.8% (10.0–28.6) and 28.8% (15.3–40.2). An independent population of otherwise similar but tamoxifen-treated patients was not tested. 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.[67] 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

Currently, neither NCCN nor ASCO recommend THEROS Breast Cancer Index[SM] as an option when evaluating breast cancer patients for risk of recurrence.

The Molecular Grade Index (Aviara MGI^{SM})

Description

The Molecular Grade Index (Aviara MGI^{SM}) 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 the MGI

Ma et al. evaluated MGI along with Aviara H/I^{SM} in a total of 733 patients[66]. High MGI was associated with significantly worse outcome only in patients with high Aviara H/I^{SM} 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 recommend The Molecular Grade Index (Aviara MGI^{SM}) 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™

- One study reports the development of the assay but provides no information on technical performance (analytic validity).[68] 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).
- An additional study of the same trial samples used for Oncotype DX® validation (NSABP B-14 and B-20 trials) 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.[69] 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).
- 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.[70]

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

Clinical Practice Guidelines

Currently, neither NCCN nor ASCO recommend Mammostrat™ as an option when evaluating breast cancer patients for risk of recurrence.

BreastOncPx™

Description

The BreastOncPx™ test is an 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.[71] No information on analytic validity was provided. In order to develop a gene signature that was completely prognostic for distant recurrence and not confounded by treatment prediction, samples from untreated patients with early breast cancer were used. The training set (n=142) was derived from a cohort diagnosed with lymph node-negative, stage T1 and T2 breast cancer from 1975 to 1986; ER-positive samples from patients who had had no systemic treatment were selected for analysis. Fourteen genes were eventually selected as most prognostic of time to distant metastasis and were given equal weighting in a summary metastasis score (MS). Using a single cutoff, patients are separated into high and low risk groups.

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

  ROC analysis of the continuous MS for distant metastasis and for death at 10 years, compared to Adjuvant!, resulted in slightly higher 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 recommend BreastOncPx™ as an option when evaluating breast cancer patients for risk of recurrence.

NexCourse® Breast IHC4

NexCourse Breast IHC4 evaluates the protein expression of ER/PR, HER2, and Ki-67 to provide a combined recurrence risk score. The assay technology uses quantitative image analysis to measure immunofluorescent signals, with results that can be combined in an algorithm to generate the recurrence risk score. The use of quantitative immunofluorescence is said to increase sensitivity, be more reproducible, and allow specific measurement of tumor cells.[72,73]

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.[74]. Of these, 793 were node-negative and 59 were HER2-positive (but were not treated with trastuzumab). A prognostic model that combined the 4 immunohistochemical markers was created (IHC4). In a model combining either IHC4 or Oncotype DX Rs with classical prognostic variables, the IHC4 score was found to be similar to the Oncotype DX RS, and little additional prognostic value was seen in the combined use of both scores. In a direct comparison the IHC4 score was modestly correlated with the Oncotype DX RS (r=0.72); the correlation was similar for node-negative patients (r=0.68). As an example, for a 1-2 cm, node-negative poorly differentiated tumor treated with anastrozole, 9-year distant recurrence at the 25th versus 75th percentiles for IHC4 and Oncotype DX were 7.6% versus 13.9% and 9.2% versus 13.4%, respectively. The IHC4 score was validated in a separate cohort of 786 ER-positive women, about half of whom received no endocrine treatment. The IHC4 score was significant for recurrence outcomes (HR: 4.1; 95% CI: 2.5-6.8).

Barton et al. assessed the clinical utility of IHC4 plus clinicopathologic factors (IHC4 + C) by comparison with Adjuvant! Online and the Nottingham Prognostic Index (NPI)[75]. The study prospectively gathered clinicopathologic data for consecutively treated postmenopausal patients (n=101 evaluable) with hormone receptor-positive, HER2-negative, LN-negative or -positive with 1-2 nodes, resected early breast cancer. Of 59 patients classified as intermediate-risk group by the NPI, IHC4 reclassified 24 to low risk and 13 to high risk. IHC4 reclassified 13 of 32 Adjuvant! high-risk patients to intermediate risk, and 3 of 32 to low risk. In addition, 15 of 26 Adjuvant! intermediate-risk patients were reclassified to low risk. No Adjuvant! low-risk patients were reclassified high risk.

PAM50 Breast Cancer Intrinsic 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.

Studies of PAM50 Breast Cancer Intrinsic Classifier

- The initial development of the PAM50 Breast Cancer Intrinsic Classifier was reported by Parker et al.[76] In an independent test set, the test using three categories of risk (low, intermediate, and high) was significantly prognostic (Log-rank p=0.0002).
- 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.[77] 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.

- Cheang et al. determined PAM50 intrinsic subtypes for samples from a clinical trial randomizing premenopausal women with node-positive breast cancer to 2 different regimens of chemotherapy. The PAM50 intrinsic subtype for 476 tumors was correlated to relapse-free survival (RFS; p=0.0005) and overall survival (OS: p<0.0001).[78] 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 OS). 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.

Clinical Practice Guidelines

Currently, neither NCCN nor ASCO recommend PAM50 Breast Cancer Intrinsic Classifier as an option when evaluating breast cancer patients for risk of recurrence.

BluePrint™ and TargetPrint®

- The BluePrint molecular subtyping profile was developed using 200 breast cancer specimens that had concordant ER, PR and HER2 protein levels by immunohistochemistry and TargetPrint mRNA readout.[79] Using a threefold cross validation procedure, the 80 genes thought to best discriminate the three molecular subtypes were identified. BluePrint was confirmed on 4 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.
- 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).[80] 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.

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[32,81] (updated in 2014)[82] 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.

BreastPRS

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.[83] BreastPRS is a binary assay which stratifies patients into low- and high-risk groups.[84]

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.[84] The authors compared the BreastPRS prognostic index to the OncotypeDX 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 OncotypeDX 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 OncotypeDX 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 OncotypeDX cases, 27 (90%) were classified as high-risk by BreastPRS, and 95 low-risk OncotypeDX 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 OncotypeDX 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 recommend BreatPRS as an option when evaluating breast cancer patients for risk of recurrence.

EndoPredict

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.[85] 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 9 patients as high-risk. There were major discrepancies in 6 of 34 cases (18%), with low-risk RS classified as high-risk by EP in 6 cases. When the RS intermediate and high-risk groups were combined, the concordance between both tests was 76%. The clinical relevance of these discrepant test results with respect to outcome is unknown.

Clinical Practice Guidelines

Currently, neither NCCN nor ASCO recommend EndoPredict as an option when evaluating breast cancer patients for risk of recurrence.

Test Comparison Studies

- Dowsett et al. compared the risk of recurrence (ROR) score generated by PAM50 to the OncotypeDx 21- gene recurrence score (RS), four immunohistochemical markers (IHC4) for ER, PR, Ki67 and HER2, and a clinical treatment score (CTS). (77) Patients had ER-positive, primary breast disease treated with anastrozole or tamoxifen in the ATAC trial (a double-blinded, phase 3 clinical trial that was designed to compare the ability of anastrozole, tamoxifen, and the 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 4 subgroups: lymph node negative, lymph node positive, HER2 negative and HER2 negative/node-negative, and that more information was added by ROR than RS. More patients scored as high risk of recurrence and fewer as intermediate risk by ROR than RS.
- 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.[86] Fifty-six articles published original evidence addressing the 21-gene recurrence score [OncotypeDX] (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 OncotypeDx 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. Mammostrat 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.
- 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.[87] The classifiers included the 21-gene Recurrence Score, the 70-gene signature, the H/I ratio, and the intrinsic subtype classifier (similar to the commercially available PAM50). Most highly correlated were the 21-gene Recurrence Score and the 70-gene signature at a Cramer's V of 0.6 (scale 0 to 1 with 1 indicating perfect agreement). More specifically, 81 of the 103 samples with a Recurrence Score of low or intermediate risk were classified as having a low risk 70-gene profile. Restricting the analysis to the 225 ER-positive samples slightly reduced the correlation. The analysis was not further restricted to node-negative patients, the present indication for both tests.

- Espinosa et al. compared the 21-gene Recurrence Score (Oncotype DX®), the 70-gene signature (MammaPrint®), and the 2-gene ratio (H/I Ratio) in 153 patients with ER-positive breast cancer treated with adjuvant tamoxifen.[42] Thirty-eight percent of these patients were node-positive, and 63% were additionally treated with chemotherapy. Distant metastasis-free survival for the Recurrence Score profile was 98% for low-risk patients versus 81% intermediate risk versus 69% high-risk; for the 70-gene signature the estimates were 95% good prognosis versus 66% poor prognosis; and for the 2-gene ratio, 86% favorable versus 70% unfavorable. There was a good correlation between the 21-gene Recurrence-Score and the 70-gene signature (Cramer's V=0.6). Slightly more variation in distant metastasis-free survival was explained by the combination of the 21-gene Recurrence score and either Adjuvant! Online (25.8+1.4) or the Nottingham Prognostic Index (NPI; 23.7+1.5) than by the combination of the 70-gene signature with Adjuvant! Online (23.1+1.2) or the NPI (22.4+1.3) but the differences were very small and any combination was significantly better than any test or clinicopathologic classifier alone.

Two recent papers compared the Oncotype DX and other gene expression profiles. Kelly et al. (74) evaluated Oncotype DX and PAM50 in 108 cases and found good agreement between the 2 assays for high- and low-prognostic risk assignment, but PAM50 assigned about half of Oncotype DX intermediate-risk patients to the PAM50 luminal A (low risk) category.[88] 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).[21]

Summary

<u>Oncotype DX®</u>

Oncotype DX® Assay in Node-Negative Patients

Despite the lack of evidence of clinical utility from randomized controlled trials, Oncotype DX® scoring has become an accepted standard of care when evaluating patients without lymph node involvement. The use of Oncotype DX® assay in node-negative population is supported by strong evidence of clinical validity and limited, but sufficient, evidence of analytic validity. The Oncotype DX® recurrence score (RS) is strongly associated with risk of distant recurrence in women with breast cancer that is positive for hormone receptors, negative for HER2, and without lymph node involvement. The assay adds additional risk information to the conventional clinical classification of individual high-risk patients, and identifies a subset of patients who would otherwise be recommended for chemotherapy but who are actually at lower risk of recurrence. Thus, a woman who prefers to avoid the toxicity and inconvenience of chemotherapy and whose Oncotype DX® RS value shows that she is at very low risk of recurrence might reasonably decline chemotherapy. In addition, National Comprehensive Cancer Network (NCCN) guidelines consider Oncotype DX® an option when evaluating patients without 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 no evidence from randomized controlled trials (RCTs) demonstrating clinical utility of Oncotype DX® testing in the node-positive population. In addition, the use of this assay in node-positive breast cancer patients is not supported by strong evidence of clinical validity comparable to the evidence available for node-negative patients. For women with node-positive breast cancer, it is less clear that the risk of recurrence in low-risk RS patients is sufficiently low, or that the benefit of chemotherapy is insufficiently large, to recommend avoiding otherwise currently recommended treatment. In addition, NCCN guidelines note that patient selection for assay use in lymph node-positive patients remains controversial. Additional studies of Oncotype DX® in lymph node-positive patients are necessary and ongoing. Therefore, Oncotype DX® testing in node-positive patients is considered investigational.

Oncotype DX® Assay in DCIS Patients

For women with ductal carcinoma in situ (DCIS), studies on the use of Oncotype DX DCIS to predict recurrence and inform treatment planning post-excision have not been published. Moreover, no information is yet available on whether women are better categorized as to their recurrence risk by the Oncotype DX DCIS Score compared with standard clinical indicators of risk; therefore Oncotype DX DCIS is considered investigational.

Oncotype DX® Assay in Men

For men with primary breast cancer, studies of the utilization of Oncotype DX to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy have not been published. Further, NCCN guidelines do not address Oncotype DX utilization in men. Therefore, use of the Oncotype DX assay in men is considered investigational.

Oncotype DX® Assay to Determine or Confirm HER2 Status

Guidelines for confirmation of HER2 status specify IHC and/or fluorescence in situ hybridization (FISH) as the recommended testing methods.[32] The HER2 component of the 21-gene assay has been shown in one large study to strongly correlate with FISH results but significant discrepancies have been noted in another.[33,89] As a result, and without evaluation and support from guidelines, it has been recommended that the 21-gene assay not be ordered to determine or confirm HER2.[90] Therefore, use of the Oncotype DX assay to determine or confirm HER2 status is considered investigational.

MammaPrint®

A large number of studies of clinical validity, and a few attempting to address the clinical utility of the MammaPrint® (70-gene signature) have been published. Several studies have pooled and re-analyzed subsets of previously published data in attempts to arrive at more homogeneous sample populations. Nevertheless, the studies of the 70-gene signature continue to suffer from confounding in heterogeneous sample populations. Pooled re-analyses of subpopulations may control for one variable (e.g. nodal status), but confounding remains from other variables (e.g. treatment heterogeneity). Results for the 70-gene signature good prognosis patients have confidence intervals that extend into ranges that likely confer too much risk for patients and providers in the U.S. Because the test result is not a continuous numerical value, patients cannot view their result within the spectrum of good prognosis results and

adjust their preferences accordingly. Therefore, MammaPrint® testing is considered investigational.

Breast Cancer Index℠, Molecular Grade Index (Aviara MGI℠), Mammostrat™, BreastOncPx™,PAM50 Breast Cancer Intrinsic Classifier™, NexCourse® Breast IHC4, BreastPRS™, and EndoPredict™

The available evidence supporting the Breast Cancer Index℠, Molecular Grade Index (Aviara MGI℠), Mammostrat™, BreastOncPx™, PAM50 Breast Cancer Intrinsic Classifier™, NexCourse® Breast, BreastPRS™, and EndoPredict™ IHC4 tests is limited in quantity and quality. No clear and reliable evidence supporting clinical utility is available for these tests. In addition, neither the National Comprehensive Cancer Network (NCCN) nor the American Society of Clinical Oncology (ASCO) guidelines currently recommend these tests as an option when evaluating breast cancer patients for risk of recurrence. Therefore, Breast Cancer Index℠, Molecular Grade Index (Aviara MGI℠), Mammostrat™, BreastOncPx™, PAM50 Breast Cancer Intrinsic Classifier™, NexCourse® Breast, BreastPRS™, and EndoPredict™ IHC4 tests are considered investigational.

BluePrint® and TargetPrint®

There is insufficient evidence demonstrating the clinical utility of the gene expression assays BluePrint® and TargetPrint®, and it is uncertain how results guide treatment decisions for patients (men or women) with breast cancer. Neither the National Comprehensive Cancer Network (NCCN) nor the American Society of Clinical Oncology (ASCO) recommends BluePrint® or TargetPrint® as options when evaluating breast cancer patients for risk of recurrence. Therefore, the gene expression assays BluePrint® and TargetPrint® are considered investigational for all indications.

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CROSS REFERENCES

Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20

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

CODES	NUMBER	DESCRIPTION
CPT		No specific CPT codes
HCPCS	S3854	Gene expression profiling panel for use in the management of breast cancer treatment