Regence

Medical Policy Manual Genetic Testing, Policy No. 42

Assays of Genetic Expression in Tumor Tissue as a Technique to Determine Prognosis in Patients with Breast Cancer

Effective: April 1, 2025

Next Review: December 2025
Last Review: February 2025

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 evaluating the potential benefit from adjuvant therapies. 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.

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 Recurrence Score, Breast Cancer Index™, MammaPrint®, or EndoPredict® 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 primary breast cancer, stage I, II, or III (see Policy Guidelines);

   B. 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, however biopsy sample testing after full pathologic evaluation may be indicated in rare circumstances when tumor testing is not possible);

C. Primary tumor size greater than 0.5 cm;

D. Hormone receptor positive (that is ER-positive or PR-positive, see Policy Guidelines);

E. HER2-negative (see Policy Guidelines);

F. Individual has negative lymph nodes or 1 to 3 positive lymph nodes (nodes with micrometastases of 2 mm or smaller are considered node negative); and

G. Individual has not already made the decision to undergo or forego chemotherapy.

II. The use of Breast Cancer Index™ to determine recurrence risk, for deciding whether or not to receive extended endocrine therapy (beyond 5 years), may be considered **medically necessary** when all of the following criteria are met:

A. Individual has primary breast cancer, stage I, II, or III (see Policy Guidelines);

B. 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, however biopsy sample testing after full pathologic evaluation may be indicated in rare circumstances when tumor testing is not possible);

C. Primary tumor size greater than 0.5 cm;

D. Hormone receptor positive (that is ER-positive or PR-positive, see Policy Guidelines);

E. HER2-negative (see Policy Guidelines);

F. Individual has negative lymph nodes or 1 to 3 positive lymph nodes (nodes with micrometastases of 2 mm or smaller are considered node negative); and

G. Individual has not already made the decision to undergo or forego extended endocrine therapy.

III. Use of Oncotype DX® Breast Recurrence Score, Breast Cancer Index™, MammaPrint®, or EndoPredict® on surgical tumor specimens to determine recurrence risk in patients with primary breast cancer is considered **not medically necessary** for patients who do not meet Criterion I. or II. above.

IV. All other uses of gene expression assays for breast cancer are considered **investigational**, including but not limited to:

A. Use of Oncotype DX® Breast Recurrence Score, Breast Cancer Index™, MammaPrint®, or EndoPredict® for predicting response to specific chemotherapy regimens or determining HER2 status.

B. Use of other assays of genetic expression in breast tumor tissue, including but not limited to BluePrint®, Mammostrat®, TargetPrint®, Oncotype Dx Breast DCIS Score, and Prosigna™/PAM50.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

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

Hormone receptor and HER2 status may be determined from needle core biopsy or from the full pathological evaluation.

LIST OF INFORMATION NEEDED FOR REVIEW

REQUIRED DOCUMENTATION:

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 variants 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, including full pathological report of excised breast mass

CROSS REFERENCES

1. Genetic Testing for Hereditary Breast and/or Ovarian Cancer and Li-Fraumeni Syndrome, Genetic Testing, Policy No. 02
2. Gene Expression-Based Assays for Cancers of Unknown Primary, Genetic Testing, Policy No. 15
3. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20
4. Gene Expression Profiling for Melanoma, Genetic Testing, Policy No. 29
5. Evaluating the Utility of Genetic Panels, Genetic Testing, Policy No. 64
6. Circulating Tumor DNA and Circulating Tumor Cells for Management (Liquid Biopsy) of Solid Tumor Cancers, Laboratory, Policy No. 46
7. Investigational Gene Expression and Multianalyte Testing, Laboratory, Policy No. 77

BACKGROUND

For patients 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, those with the best prognosis have small tumors, are estrogen receptor (ER)-positive, and lymph node-negative. These individuals 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 chemotherapy than can benefit. Better predictors of baseline risk could help patients 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 those with node-negative disease. The available gene expression tests include:

- Oncotype DX® Breast Recurrence Score (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® (Clarient Diagnostic Services)
- Molecular Grade Index (Aviara MGI℠; AviaraDx, Inc.)
- Breast Cancer Index™, 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)
- BreastPRS™ (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 patients 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, 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®.

EVIDENCE SUMMARY

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 ER, PR and 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 (one to three 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 Index™, and Prosigna™/PAM50 gene expression assay.[1] This report did 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 Index™, 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.

Since the TEC Assessment above, many studies have been published that have evaluated GEP testing for a variety of indications. This evidence review focuses on studies presenting a minimum of five-year distant disease recurrence rates, as well as recently published prospective studies specifically designed to evaluate the clinical utility of genetic expression profiles. Studies in which the gene expression algorithm was being developed ("training sets"), studies using convenience samples of patients, and observational studies based on registry data were not included.

ONCOTYPE DX® BREAST RECURRENCE SCORE

Oncotype DX® Breast Recurrence Score is available only from the CLIA-licensed Genomic Health laboratory as a laboratory-developed service, as it has not been cleared or approved by the FDA. 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.[2]

Oncotype DX® RS for Adjuvant Chemotherapy Decisions 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.[1] 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] Additional studies have evaluated the association between RS and recurrence risk in node-negative patients.[4-7] Results indicate strong, independent associations between Oncotype DX® RS results and distant disease recurrence or death from breast cancer.[6, 8]

Sparano (2018) conducted a RCT, Trial Assigning Individualized Options for Treatment (TAILORx), to evaluate risk of recurrence in women with midrange scores.[9] Women with intermediate-risk scores were randomized to receive either endocrine therapy (n=3,399) or chemoendocrine therapy (n=3,312). Women with low-risk scores (≤10) received endocrine therapy (n=1,619) and women with high-risk scores (≥26) received chemoendocrine therapy (n=1,389). Overall disease-free survival (DFS) estimates showed that adjuvant endocrine therapy was noninferior to chemoendocrine therapy in women with intermediate-risk scores (DFS 83.36% vs. 84.3%, respectively). However, subgroup analyses by age showed women younger than 50 may benefit from chemotherapy.

In secondary analyses of data published by Paik (2004), patient risk levels were individually classified by conventional risk classifiers, and then reclassified by Oncotype DX®.[4] Oncotype DX® added additional risk information to the conventional clinical classification of individual high-risk patients, and identified a subset of patients who would otherwise be recommended for chemotherapy, but are actually at lower risk of recurrence (average 7% to 9% risk at 10 years, upper 95% confidence interval [CI] limits 11% to 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 chemotherapy will not provide net benefit; outcomes are improved by avoiding chemotherapy toxicity.

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®.[2] 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% to 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.

Several nonrandomized studies reporting on the use of the 21-gene assay in lymph-node negative patients have been published[16, 17], including a prosepective study by Sparano (2015) that assigned women with a recurrence score of 0 to 10 to receive endocrine therapy without chemotherapy.[18] At five-years follow-up, 1,626 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). Kizy (2017) evaluated the use of the of Oncotype DX® in women with invasive lobular carcinoma, using data from the Surveillance, Epidemiology and End Results database from 2004 to 2013.[19] There were 7,316 participants included in the study, the majority with grade I or II tumors (93%) and negative lymph nodes (85%). The RS cutpoints used for most of the analyses were 11 and 25, values used in the Trial Assigning Individualized Options for Treatment (TAILORx) to avoid undertreatment. Using

these conservative cutpoints, 8% of the participants were categorized as high-risk, and 72% as intermediate risk. Adjuvant chemotherapy was not associated with any increased five-year BCSS in these high- and intermediate-risk groups.

Several studies have been published regarding the impact of RS results on chemotherapy recommendations by medical oncologists.[20-28] According to these studies, comparing recommendations made prior to and revised after knowledge of RS results show that decisions change in about 25-61% of patients, most often from endocrine therapy plus chemotherapy to endocrine therapy alone.

Oncotype DX® RS for Adjuvant Chemotherapy Decisions in Lymph Node-Positive Patients

In a systematic review partly funded by Genomic Health, Brufsky (2014) [29] assessed articles and abstracts, that evaluated the 21-gene breast cancer profiling assay (using RT-PCR technology) in patients with ER+ and node-positive early-stage breast cancer. Study results suggested that the 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 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.

Kalinsky (2021) reported results from the RxPONDER RCT.[30] Participants with hormone-receptor–positive, HER2-negative breast cancer, one to three positive axillary lymph nodes, and a RS of 25 or lower were randomized to endocrine therapy only or to chemotherapy plus endocrine (chemoendocrine) therapy. The primary objective was to determine the effect of chemotherapy on invasive disease–free survival and whether the effect was influenced by the RS. Secondary end points included distant relapse–free survival.

Among postmenopausal women, estimates of invasive disease–free survival at five years were 91.3% in the chemoendocrine group and 91.9% in the endocrine-only group (hazard ratio [HR] 1.02 for invasive disease recurrence, new primary cancer [breast cancer or another type], or death, 95% CI 0.82 to 1.26, p=0.89). In premenopausal women, the rate of invasive disease–free survival at five years among those in the chemoendocrine group was 93.9%, as compared with 89.0% among those in the endocrine-only group (absolute difference, 4.9 percentage points), with a significant chemotherapy benefit (HR 0.60 for invasive disease recurrence, new primary cancer [breast cancer or another type], or death, 95% CI 0.43 to 0.83, p=0.002). The study authors concluded that postmenopausal women with one to three positive axillary lymph nodes and a recurrence score of 0 to 25 could “safely forgo adjuvant chemotherapy without

compromising invasive disease–free survival and distant relapse–free survival." In contrast, premenopausal women with one to three positive lymph nodes "had a significant benefit from chemotherapy, even with a very low recurrence score." A follow-up study by Abdou (2024) found that non-Hispanic Black participants in the study had worse clinical outcomes that non-Hispanic White participants, despite having similar RS scores and similar treatment.[31]

Nitz (2017) conducted a phase 3 Plan B trial with a mixed population of women with node-negative and node-positive breast cancer.[32] The trial was initially designed to compare anthracycline-containing chemotherapy with anthracycline-free therapy. An amendment was made to recommend endocrine therapy alone for patients with an RS of 11 or less that were node-negative or had only one positive node. A total of 2,642 patients were included in the trial. Median age was 56 years, 59% were node-negative, 35% had one positive nodes, and 6% had two or three positive nodes. Details of subgroup analyses of node-positive patients were limited. The authors stated that five-year overall survival in patients with an RS between 12 and 25 was significantly higher than in patients with an RS greater than 25 within all nodal subgroups and that five-year overall survival in low RS patients was higher compared with high RS patients in all nodal subgroups, but rates and CIs were not provided. Five-year DFS in patients with one positive node and a RS ≤11 treated with endocrine therapy alone (n=110) was 94.4% (95% CI 89.5 to 99.3%). The final analysis of the Plan B trial reported similar results regarding RS scores and DFS.[33]

Albain (2010) published retrospective analysis of the OncotypeDX® assay.[34] 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, regardless 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 overall survival.

Additional Applications of Oncotype DX®

In 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 that compared the Oncotype DX® ER and PR results with traditional immunohistochemistry (IHC) results.[35] 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, when the patient meets specific criteria and chemotherapy is being considered. 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, so the 21-gene RS should not be ordered to confirm ER/PR IHC results. A subsequent study by Khoury (2015) reported better correlation between IHC and Oncotype DX® for PR (Spearman correlation, 0.91) than for ER (Spearman correlation, 0.65), but worse concordance (at various cutpoints) for PR than for ER (99% vs 88%, respectively).[36]

Similarly, guidelines for HER2 testing specify IHC and/or FISH methods.[37] Although the HER2 component of the 21-gene assay has been shown to strongly correlate with FISH results,[38] the 21-gene assay should not be ordered to determine or confirm HER2.

MAMMAPRINT®

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.

Mammaprint® for Adjuvant Chemotherapy Decisions

The Microarray In Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy (MINDACT trial) published by Cardoso (2016), was a prospective trial that 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.[39] Women at low risk according to both methods did not receive chemotherapy. Women with discordant risks were randomized to chemotherapy or to no chemotherapy. Women at high-risk with both methods received chemotherapy. Although there were randomized components of the study, the primary endpoint was a noninferiority outcome of five-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. Declaring this to be the main end point implies a clinical strategy of using MammaPrint® only in patients at high clinical risk, and deferring chemotherapy in those tested patients who have low genetic risk scores. In this strategy, patients at low clinical risk are not tested with MammaPrint®.

While trial entry criteria included patients with node-positive, estrogen receptor-negative, or *HER2*-positive breast cancer, these patients constituted a minority of those in the study. The main results included these patients. The authors conducted supplemental analyses of various subgroups, including the subset who were node-negative, estrogen receptor-positive, or *HER2*-negative, which were qualitatively similar to the published main results.

In the main article, the principal objective of the study was met. The group at high clinical risk and low genomic risk who did not receive chemotherapy had a distant recurrence rate of 5.3% (95% CI 3.8% to 7.5%). In the node-negative, estrogen receptor-positive, or *HER2*-negative subgroup analysis, this group had a distant recurrence rate of 4.5% (95% CI, 3.8% to 8.4%). Piccart (2021) reported updated results from MINDACT with a median follow-up of 8.7 years.[40] In the updated analysis, five-year distant metastasis-free survival rate for individuals with high clinical risk and low genomic risk receiving no chemotherapy (primary test population, n=644) was 95.1% (95% CI 93.1% to 96.6%), supporting the previous analysis.

In the group with clinical low-risk and high genomic risk, who were not considered in the main outcome, in both the main analysis and in the node-negative, estrogen receptor-positive, or *HER2*-negative subgroup, the results would indicate that the risk of distant recurrence is not low enough to avoid chemotherapy (main analysis distant recurrence 5%, 95% CI 3% to 8.2%, subgroup distant recurrence HR 6.1%, 95% CI 3.9% to 9.4%). In the testing strategy implied in this study, by not testing for genomic risk in the low clinical risk group, these patients would not be identified.

The groups randomized to chemotherapy showed no significant difference in five-year distant recurrence, but the CIs were wide and thus less informative regarding whether chemotherapy is or is not beneficial in these patient groups. In the main study, the HR for chemotherapy in the high clinical risk/low genomic risk was 0.78 (95% CI 0.5 to 1.21). The HR for chemotherapy in the low clinical risk/high genomic risk group was 1.17 (95% CI 0.59 to 2.28).

To assess the impact of MammaPrint® on treatment decision-making, Cusumano (2014) distributed clinical information on 194 patients to multidisciplinary teams initially without and then with MammaPrint® gene signatures.[41] 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.

Mammaprint® for Extended Endocrine Therapy Decisions

Esserman (2017) conducted a secondary analysis on data from women who were node-negative, in the Stockholm tamoxifen trial, which randomized patients with node-negative breast cancer to two years of tamoxifen, followed by an optional randomization for an additional three years to tamoxifen or no treatment.[42] A total of 652 tissue samples from the trial underwent MammaPrint® risk classification, 313 from the tamoxifen arm and 339 from the no therapy arm. The primary outcome was 20-year breast cancer-specific survival (BCSS). Initial classification by MammaPrint® identified 58% of the patients as low risk for distant recurrence and 42% as high risk. Twenty-year BCSS rates were 85% and 74% ($p<0.001$), respectively. Analysis was conducted on a subgroup of the low-risk group, considered ultralow risk. The tamoxifen-treated ultralow-risk group did not experience any deaths at 15 years. Survival rates were high for all patients in the ultralow-risk group, 97% for those treated with tamoxifen and 94% for those untreated.

BREAST CANCER INDEX™ (BCI)

The Breast Cancer Index™ 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.

Breast Cancer Index™ for Adjuvant Chemotherapy Decisions

The 2014 TEC Assessment reviewed available studies for the original component assays.[1] There was insufficient evidence to determine whether the H/I ratio is better than conventional risk assessment tools in predicting recurrence. The ten-year recurrence estimates for patients classified as low risk were 17% to 25%, likely too high for most patients and physicians to consider forgoing chemotherapy.

Schroeder (2017)[43] calculated distant recurrence-free survival rates following five years of endocrine therapy among the subset of patients with clinically low-risk (T1N0) breast cancer from the two populations studied by Zhang (2013), described below. The Stockholm trial had 237 patients, and the U.S. medical center cohort contributed 210 patients that were T1N0. BCI classified 68% (160/237) and 64% (135/210) of the Stockholm population and the medical

center population as low risk, respectively. Median follow-up was 17 years for the Stockholm study and 10 years for the medical center cohort. Among the BCI high-risk, HER2-negative participants, the 5- to 15-year distant recurrence-free survival rates in the Stockholm trial and the multi-institutional study were 86.9% (95% CI 78.8% to 95.9%) and 87.5% (95% CI 79.1% to 96.9%), respectively. The rates in the low-risk, HER2-negative groups were 95.2% (95% CI 91.9% to 98.8%) and 98.4% (95% CI 96.1% to 100%), respectively.

A retrospective study by Sgroi (2016) 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.[44] 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 lymph node-positive patients received chemotherapy, the prognostic utility of BCI could not be assessed for those patients.

Zhang (2013) evaluated a continuous risk model derived from the H/I ratio and MGI in tumor samples from 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.[45] An optimized continuous recurrence risk model, the Breast Cancer Index™ 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-positive samples and the multicenter registry included 12% HER2-positive 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.

Breast Cancer Index™ for Endocrine Therapy Decisions

Sgroi (2013) examined 665 lymph node-negative, ER-positive, postmenopausal women receiving endocrine therapy but no chemotherapy in the ATAC trial.[46] 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 (2013) 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).[46] 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).

Sgroi (2013) conducted a prospective-retrospective, nested case-control study within the

MA.17 trial that compared extended endocrine therapy (letrozole) with placebo in postmenopausal women who had hormone receptor-positive cancers.[47] The trial randomized 5,157 women recurrence-free at five years to letrozole or placebo. A case-control design was adopted owing to challenges in obtaining archived tumor samples. An eligible case (319 of which 83 were examined) was one that experienced a local, regional, or distant recurrence and had an available tumor sample. Two controls free of recurrence longer than cases were matched to each case based on age, tumor size, node status, and prior chemotherapy. Any recurrence (locoregional or distant) was used as the endpoint; patients with contralateral or unknown recurrences were excluded. Using the BCI H/I ratio, there was a 42% relative risk reduction in the low-risk group vs. a 77% reduction in the high-risk group. Although statistical significance was lacking in the low-risk group, the CIs were wide and included values consistent with those observed in the high-risk group. The Zhang (2013) study described above,[45] as well as studies by Bartlett (2019)[48] and Noordhoek (2021)[49] also reported a larger potential relative risk reduction with extended endocrine therapy in the H/I high-risk group, with similar uncertainty reflected in the CIs (HR 0.35, 95% CI 0.19 to 0.65; HR 0.35, 95% CI 0.15 to 0.86; and HR 0.34, 95% CI 0.16 to 0.73, respectively).

ONCOTYPE DX® 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.[50]

In a retrospective analysis, Rakovitch (2015) evaluated 571 tumor specimens with negative margins from a convenience cohort of patients with DCIS treated by breast-conserving surgery (lumpectomy) alone.[51] Patients were drawn from a registry of 5752 women in Ontario, Canada, who were diagnosed with DCIS between 1994 and 2003. Median follow-up of the 571 women was 9.6 years. There were 100 local recurrence events (18% prevalence); 43 were DCIS (8% prevalence), and 57 were invasive cancer (10% prevalence). Oncotype DX® DCIS score was significantly associated with local recurrence outcomes (HR 2.15, 95% CI 1.43 to 3.22). Sixty-two percent of patients were classified as low-risk, 17% as intermediate risk, and 21% as high risk. Corresponding 10-year local recurrence estimates were 13% (95% CI 10% to 17%), 33% (95% CI 24% to 45%), and 28% (95% CI 20% to 38%), respectively. Corresponding 10-year estimates for DCIS recurrence (5%, 95% CI 3% to 9%; 14%, 95% CI 8% to 24%; 14%, 95% CI 9% to 22%; respectively) and for invasive breast cancer recurrence (8%, 95% CI 6% to 12%; 21%, 95% CI 13% to 33%; 16%, 95% CI 9% to 25%; respectively) were based on small numbers of events. It is unclear whether estimated recurrence risks for patients classified as low risk are low enough to forgo radiotherapy.

In a retrospective analysis of data and samples from patients in the prospective Eastern Cooperative Oncology Group E5194 study by Solin (2013), 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).[52] Oncotype DX® DCIS Score was significantly associated with recurrence outcomes (HR 2.31, 95% CI 1.15 to 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.

Rakovitch (2018) combined the populations from the two studies described above (Solin [2013] and Rakovitch [2015]) and calculated 10-year local recurrence rates by DCIS category (low, intermediate, and high), age, tumor size, and year of diagnosis.[53] Ten-year recurrence rates in the low risk score group ranged from 7.2% (95% CI 5.3% to 10.0%) for those age 50 and above with tumors ≤1 cm to 11.6% (95% CI 7.7% to 15.5%) for those with tumors > 2.5 cm.

DCISIONRT®

The DCISionRT test combines seven monoclonal protein markers (COX-2, FOXA1, HER2, Ki-67, p16/INK4A, PR, and SIAH2) assessed in tumor tissue with four clinicopathologic factors (age at diagnosis, tumor size, palpability, and surgical margin status) to produce a score that stratifies individuals with DCIS into three risk groups: low risk, elevated risk with good response, and elevated risk with poor response. The purpose of the test is to predict radiation benefit in individuals with DCIS following breast conserving surgery.

Warnberg (2021) analyzed the association of DCIS RT score with risk of recurrence in 504 individuals with DCIS enrolled in the SweDCIS randomized trial.[54] This study is Simon Category B. Using a cutoff of DS >3, 52% of participants were categorized as elevated risk and 48% as low risk. In the low-risk group, there was no significant difference in risk of recurrence observed with radiotherapy. In contrast, radiotherapy was associated with reduced risk of total and invasive ipsilateral recurrence in the elevated-risk group.

PROSIGNA™/ PAM50 BREAST CANCER INTRINSIC SUBTYPE CLASSIFIER

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[55] in place of qRT-PCR to assay 46 genes instead of the original 50. The 2014 TEC Assessment reviewed development and validation studies of the PAM50 intrinsic subtype classifier and Prosigna™.[1]

In a study that supported FDA clearance of Prosigna™, Gnant (2014) evaluated tumor samples from 1,047 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.[56] ABCSG-8 randomized hormone receptor-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.

Dowsett (2013) reported on groups from the ATAC trial stratified by subtype (luminal A or B) and by PAM50 ROR class, both with and without consideration of clinicopathologic factors.[57] 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.

Two studies published in 2015 presented combined analyses of pretreatment FFPE tumor specimens from ABCSG-8 and ATAC trial monotherapy arms (TransATAC).[58, 59] Median follow-up was 10 years. Sestak (2015) examined the association between ROR score and late distant recurrence (5 to 10 years after diagnosis) in 2,137 postmenopausal women (60% from ABCSG-8).[58] Patients had HR–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. Cutpoints differed from cutpoints used in the FDA-approved version of the test, designed to assess recurrence risk in the first 10 years after diagnosis (years 0 to 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% to 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 to 3.5), 8.5% (95% CI 5.9 to 12.1), and 9.3% (95% CI 5.5 to 15.5), respectively. In node-positive patients, estimated risks were 3.3% (95% CI 1.2 to 8.6), 7.8% (95% CI 4.4 to 13.8), and 20.9% (95% CI 16.1 to 26.9) in low-, intermediate-, and high-risk groups, respectively. 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.

The other study, by Gnant (2015), evaluated FFPE tissue specimens from 543 patients in the ABCSG-8 and ATAC trials who had one to three positive lymph nodes.[59] The primary endpoint 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.[60] 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 cutpoints for defining risk groups differed from cutpoints used in the FDA-approved version of the test, which were defined by Gnant (2014),[56] discussed below. 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% to 12.8%), 15.5% (95% CI 9.5% to 25.0%), and 25.5% (95% CI 17.5% to 36.0%), respectively. Because the upper bound of the 95% CI for patients categorized as low risk exceeded 10%, usefulness of these risk distinctions is uncertain. For patients with 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% to 22.8%) and 33.7% (95% CI 25.5% to 43.8%), respectively. When ROR, either as a continuous or a categorical variable, was added to CTS, prognostic information was improved (changes in likelihood ratios were statistically significant) compared with CTS alone for all nodal subgroups, including node-negative patients.

Sestak (2013) reported on the prognostic ability of PAM50 ROR score in 940 (16%) of 5880 patients from the ATAC trial.[61] Thirty percent of patients were lymph node positive. Investigators modified the ROR scoring algorithm to exclude tumor size and defined cutpoints 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.

Hequet (2017)[62] and Martin (2015)[63] evaluated the impact of ROR on treatment decision making in patients with ER-positive, HER2-negative, node-negative breast cancer. Because survival or recurrence outcomes were not reported, these studies are considered uninformative for assessing clinical utility of Prosigna™.

The majority of PAM50/Prosigna™ studies suffered from confounding due to heterogeneous patient samples. It is therefore difficult to estimate outcomes for the patients of interest: ER-positive, HER2-negative, lymph node-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® AND TARGETPRINT®

Gene expression patterns have led to the identification of molecular subtypes of breast cancer, which have different prognoses and responses to treatment regimens. These molecular subtypes are largely distinguished by differential expression of ER, PR, and 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® 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®. BluePrint® classifies breast cancer into basal type, luminal type or ERBB2 type. TargetPrint® is a microarray-based gene expression

test that offers a quantitative assessment of ER, PR and HER2 overexpression in breast cancer. Both BluePrint® and TargetPrint® are intended for use with MammaPrint®. Wesseling (2016) compared TargetPrint® 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® was 96%/87% for ER, 84%/74% for PR, and 74%/98% for HER2.[64] The authors noted substantial discord in IHC/ISH results between different hospitals and indicated that TargetPrint® might improve the reliability of these discordant results by prompting retesting in a reference laboratory.

Gran (2015) compared HER2 testing results by IHC, FISH, and TargetPrint® in 127 tumor specimens from patients with early-stage breast cancer in South Africa.[65] 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.

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.[66] 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 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.

Whitworth (2014) 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.[67] 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.

Wuerstlein (2019) conducted a prospective evaluation of how MammaPrint® and BluePrint® influence clinical therapy decisions in patients with luminal early breast cancer.[68] About 72% (309 out of 430) of patients had node-negative disease. Specifically focusing on the impact of BluePrint® testing, the investigators found that there was a 65% concordance rate between IHC assessment and BluePrint® subtyping for Luminal A or B-like tumors. Notably, BluePrint® reclassified two clinically identified Luminal A-like tumors and four Luminal B-like tumors as Basal type. Additionally, BluePrint® reclassified 46% (80 out of 173) of Luminal B-like tumors to Luminal A, and 24% (62 out of 256) of Luminal A-like tumors to Luminal B. This led to an

overall discordance rate of 34% in subtype classification. The study also highlighted the strong association between chemotherapy recommendations and molecular subtype: 94% (143 out of 152) of patients with molecular Luminal B tumors received a recommendation for chemotherapy, whereas 92% (251 out of 272) of patients with molecular Luminal A tumors were advised to omit chemotherapy.

ENDOPREDICT®

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

Filipits (2011) reported on the validation of EndoPredict® using tumor samples from women receiving endocrine treatment in the ABCSG-6 and ABCSG-8 trials.[69] 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. Filipits (2019) published a follow-up to this study, which reported outcomes for 1,702 patients and reported that patients with low-risk EPclin scores (62.6%) had increased distant recurrence-free rates compared with patients that had high-risk scores (HR 4.77, 95% CI 3.37 to 6.67), and that the EPclin scores were significantly associated with this rate regardless of nodal status.[70]

Sestak (2019) reported results of an analysis of the performance of EndoPredict® to predict chemotherapy benefit.[71] The analysis included 3,746 women; 2,630 patients received five years of endocrine therapy alone (from ABCSG-6/8, TransATAC trials) and 1,116 patients received endocrine therapy plus chemotherapy (from GEICAM 2003-02/9906 trial). There was a significant positive interaction between EPclin as a continuous measure and treatment group for the outcome of the ten-year recurrence rate (interaction p=0.022). Although the comparison is indirect, it may suggest that a high EPclin score can predict chemotherapy benefit in women with ER-positive, HER2-negative disease.

Buus (2016) evaluated EndoPredict® as a prognostic indicator for breast cancer recurrence in women treated endocrine therapy.[72] 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 to 6.0) for the EP low group and 14.5% (95% CI 11.3 to 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 to 8.6) and 20.0% (95% CI 14.6 to 27.0), respectively. The 10-year recurrence rates were also determined for node-positive patients: 21.3% (95% CI 13.9 to 31.9) for the EP low group, 36.4% (95% CI 29.6 to 40.1) for the EP high group, 5.0% (95% CI 1.2 to 18) for the EPclin low group, and 36.9% (95% CI 30.2 to 44.5) for the EPclin high group.

Martin (2014) assessed tumor samples from 566 ER-positive, HER2-negative patients who participated in the GEICAM 9906 RCT.[73] GEICAM 9906 compared two adjuvant chemotherapy regimens in 1,246 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 to 9.6, log-rank test p<0.001). Thirteen percent (n=74) of samples were classified as low-risk by EPclin score, and 87% (n=481) were classified as high-risk; 10-year metastasis-free survival was 100% in the low-risk group and 72% in the high-risk group.

EndoPredict® for Endocrine Therapy Decisions

Dubsky (2013) examined predictive ability of EP and EPclin for early (within five years) and late (more than five years post-diagnosis) disease recurrence.[74] Tumor samples from chemotherapy-untreated, ER-positive, HER2-negative patients who participated in one of two RCTs (ABCSG-6 or ABCSG-8) were assayed (total n=1,702). 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 to 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 to 7.24, log-rank test p=0.002). By EPclin, 1,066 (63%) of 1,702 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 to 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 to 14.36, log-rank test p<0.001).

EP and EPclin appear to be able to identify a group at low-risk of distant recurrence from years 5 to 10 in this prospective-retrospective study of patients untreated with adjuvant chemotherapy enrolled in the ABCSG-6 and -8 trials. However, in the Filipits (2019) study, the lower-bound of the 95% CI for the distant recurrence rate in the high-risk group falls within a range that may be clinically meaningful for decision-making about avoiding extended ET both at 5-10 years (5.9%, 95% CI 2.2% to 9.5%) and at 5 to 15 years (15.1%, 95% CI 4.0% to 24.9%).[70] These results suggest the possibility that a proportion of high-risk patients may still have been unnecessarily treated with extended ET endocrine therapy based on a gene expression profiling result. ROC statistics (area under the receiver operating characteristic curve) were reported to support incremental improvement with the EP or EPclin over Adjuvant! Online or nodal status, tumor size, or grade. However, they appeared to include EP and EPclin as continuous variables and not threshold cutoffs for those tests that would inform decisions.

TEST COMPARISON STUDIES

Sestak (2018) compared Breast Cancer Index®, Oncotype DX®, Prosigna®, and Endopredict® using samples from the TransATAC RCT.[75] The low-risk categories of all four tests exhibited both low overall 10-year distant recurrence rates and low 5- to 10-year distant recurrence rates (within the threshold of <10%). Comparatively, among those who are

considering adjuvant chemotherapy (n=591), EPclin classified the most women as low risk (n=429) compared with the other three tests which classified 318 to 365 women as low risk. Among those who are considering extended endocrine therapy (n=535), EPclin classified the most women as low risk (n=393) compared with the other three tests, which classified 292 to 351 women as low risk.

Bosl (2017) compared MammaPrint® with EndoPredict® in 48 tumor samples - 29 were node-negative and 19 were node-positive.[76] For the MammaPrint test, RNA quality was low for three samples. Of the 45 tested by MammaPrint, 17 (38%) were classified as low-risk and 28 (62%) were classified as high-risk for recurrence. Four samples were excluded from the EndoPredict® analysis because the tumors were estrogen receptor-positive or HER2-positive, which are not part of the inclusion criteria of this test. Based on the EP molecular score, eight (18%) were classified as low-risk and 36 (82%) were classified as high-risk. Based on the EPclin score, 17 (39%) were considered low-risk and 27 (61%) were considered high-risk. There was no statistically significant agreement between MammaPrint® and molecular EP (overall concordance, 63%) or between MammaPrint® and EPclin (overall concordance, 66%).

Sgroi (2013) compared the Breast Cancer Index™ and Oncotype DX® in 665 lymph node-negative women receiving endocrine therapy but not chemotherapy in the ATAC trial.[46] 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 Index™ was a better predictor of risk: 5% of Breast Cancer Index™ low-risk patients had distant recurrence compared with 9% of Oncotype DX® low-risk patients, and 22% of Breast Cancer Index™ 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.

Sestak (2016)[77] examined cross-stratification between the Breast Cancer Index™ and Oncotype DX® RS using the same data as Sgroi (2013). Gene expression analyses for both scores were conducted, and risk categories were determined based on prespecified cutoff points (RS <18: low risk, 18 to 31: intermediate risk, >31: high risk; BCI <5.0825: low risk, 5.0825 to 6.5025: intermediate risk, >6.5025: high risk). Each gene expression score was combined with the CTS an algorithm of nodal status, tumor size, grade, age, and treatment. In a multivariate analysis, when BCI was added to RS plus CTS, there was a significant effect on prognostic information. When RS was added to BCI plus CTS, no additional prognostic information was added.

Dowsett (2013) compared the PAM50 ROR score to the Oncotype DX® RS, four immunohistochemical markers (IHC4) for ER, PR, Ki67 and HER2, and a CTS.[57] 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 tests and concordance indices were used to assess the prognostic information provided beyond that of a CTS, RS, ROR or IHC4. The CTS integrated prognostic information from nodal status, tumor size, histopathologic grade, age and anastrozole or tamoxifen treatment. The authors concluded that the ROR added significant prognostic information beyond CTS in all patients ($p<0.001$), and in all 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.

The study by Buus (2016) described earlier, compared EndoPredict® with Oncotype DX® RS in hormone receptor-positive, HER2-negative tumor samples from the TransATAC study.[72] 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 within five years in node-negative disease, while EP and EPclin had more prognostic value than RS for distant recurrence in 5 to 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.

PRACTICE GUIDELINE SUMMARY

National Comprehensive Cancer Network

National Comprehensive Cancer Network (NCCN) guidelines for breast cancer (v.1.2025)[11] recommend that the 21-gene (Oncotype DX® Breast Recurrence Score) assay be strongly considered in node-negative, HR-positive, HER2-negative disease when the tumor is >0.5 cm, and of ductal/NST, lobular, mixed, or micropapillary histology (category 1), if the patient is a candidate for chemotherapy. They note that “other prognostic gene expression assays may be considered to help assess risk of recurrence but have not been validated to predict response to chemotherapy.” This test is also the preferred test for postmenopausal patients with one to three positive nodes (category 1).

MammaPrint® is also considered a category 1 option based on the results of the randomized MINDACT trial, which “demonstrated that the 70-gene assay can identify a subset of patients who have a low likelihood of distal recurrence despite high-risk clinical features (based on tumor size, grade, nodal status).” However, they note that the test is not useful for guiding chemotherapy decisions in those with low clinical risk, as no difference in outcomes with and without chemotherapy were seen in the trial for this group.

Regarding node-positive, HR-positive, HER2-negative disease, the guidelines recommend considering a multigene assay to assess prognosis and determine chemotherapy benefit for patients that are candidates for chemotherapy, The guidelines additionally state:

> “The panel notes in those with N1mi and N1 tumors, while multigene assays have yet to be proven to be predictive for adjuvant chemotherapy benefit, they are prognostic and can be used to identify low-risk patients who are likely to derive little or no absolute benefit from addition of adjuvant chemotherapy to adjuvant endocrine therapy. A secondary analysis of the prospective SWOG 8814 trial using the 21-gene assay demonstrated no benefit for chemotherapy for patients with 1-3 involved axillary lymph nodes and a low RS, and a significant benefit for the addition of adjuvant chemotherapy in those with high-RS (≥ 31). […] Other multigene assays have not proven to be predictive of chemotherapy benefit.”

Oncotype DX® is listed as the preferred multigene assay by the NCCN for node-negative disease, and predictive of chemotherapy response as well as prognostic, while the Breast Cancer Index™, Endopredict®, Prosigna®, and MammaPrint® tests were listed as prognostic only. Oncotype DX®, MammaPrint®, Prosigna®, and Endopredict® are listed as multigene assays that may be considered for individuals with one to three positive nodes, as well as those who are node negative.

The Breast Cancer Index™ is listed as being predictive of benefit of extended endocrine therapy, with evidence indicating that patients that have BCI (H/I) Low test results do not have improved survival with extending endocrine therapy beyond five years.

The guidelines do not recommend the use of multigene or mRNA assays for assignment of HER2 status.

The guidelines do not address the use of assays such as Oncotype DX® DCIS Score or DCISionRT® to guide decisions about radiation therapy in individuals with DCIS.

American Society of Clinical Oncology

In June 2022, the American Society of Clinical Oncology (ASCO) published updated clinical practice guidelines on the use of breast cancer biomarker assay results to guide adjuvant endocrine and chemotherapy decisions in early-stage breast cancer.[78] The recommendations related to the interventions and populations included in this evidence opinion include the following:

> **Newly Diagnosed ER-Positive, HER2-Negative Breast Cancer**
>
> 1.1 If a patient has node-negative breast cancer, the clinician may use Oncotype DX test to guide decisions for adjuvant endocrine and chemotherapy (Evidence Quality [EQ]: High, Recommendation Strength [RS]: Strong)
>
> 1.2. In the group of patients in Recommendation 1.1 with Oncotype DX score greater than or equal to 26, the clinician should offer chemoendocrine therapy (EQ: High, RS: Strong)
>
> 1.3. In the group of patients in Recommendation 1.1 who are 50 years of age or younger with Oncotype DX score 16 to 25, the clinician may offer chemoendocrine therapy. (EQ: Intermediate, RS: Moderate)
>
> 1.4. If a patient is postmenopausal and has node-positive breast cancer with 1-3 positive nodes, the clinician may use Oncotype DX test to guide decisions for adjuvant endocrine and chemotherapy (EQ: High, RS: Strong)
>
> 1.5. In the group of patients in Recommendation 1.4, the clinician should offer chemoendocrine therapy for those whose Oncotype DX score is greater than or equal to 26 (EQ: High, RS: Strong)
>
> 1.6. If a patient is premenopausal and has node-positive breast cancer with 1-3 positive nodes, Oncotype DX test should not be offered to guide decisions for adjuvant systemic chemotherapy (EQ: High, RS: Moderate)
>
> 1.7. If a patient has node-positive breast cancer with more than 3 positive nodes, the evidence on the clinical utility of routine Oncotype DX test to guide decisions for

adjuvant endocrine and chemotherapy is insufficient to recommend its use (EQ: Insufficient, RS: Moderate)

1.8. If a patient is older than 50 and has high clinical risk breast cancer, that is node-negative or node-positive with 1-3 positive nodes, the clinician may use MammaPrint test to guide decisions for adjuvant endocrine and chemotherapy (EQ: Intermediate, RS: Strong)

1.9. If a patient is 50 years of age or younger and has high clinical risk, node negative or node-positive with 1-3 positive nodes breast cancer, the clinician should not use the MammaPrint test to guide decisions for adjuvant endocrine and chemotherapy (EQ: High, RS: Strong)

1.10. If a patient has low clinical risk, regardless of age, the evidence on clinical utility of routine MammaPrint test is insufficient to recommend its use (EQ: Intermediate, RS: Moderate)

1.11. If a patient has node-positive breast cancer with more than 3 positive nodes, the evidence on the clinical utility of routine MammaPrint test to guide decisions for adjuvant endocrine and chemotherapy is insufficient to recommend its use (EQ: Insufficient, RS: Strong)

1.12. If a patient is postmenopausal and has breast cancer that is node negative or node-positive with 1-3 positive nodes, the clinician may use EndoPredict test to guide decisions for adjuvant endocrine and chemotherapy (EQ: Intermediate, RS: Moderate)

1.13. If a patient is premenopausal and has breast cancer that is node negative or node-positive with 1-3 positive nodes, the clinician should not use EndoPredict test to guide decisions for adjuvant endocrine and chemotherapy (EQ: Insufficient, RS: Moderate)

1.14. If a patient has breast cancer with more than 3 positive nodes, evidence on the clinical utility of routine use of EndoPredict test to guide decisions for adjuvant endocrine and chemotherapy is insufficient (EQ: Intermediate, RS: Moderate)

1.15. If a patient is postmenopausal and has breast cancer that is node negative, the clinician may use the Prosigna test to guide decisions for adjuvant systemic chemotherapy (EQ: Intermediate, RS: Moderate)

1.16. If a patient is premenopausal, and has node-negative or node-positive breast cancer the clinician should not use the Prosigna test to guide decisions for adjuvant systemic chemotherapy (EQ: Insufficient, RS: Moderate)

1.17. If a patient is postmenopausal and has node-positive breast cancer with 1-3 positive nodes, the evidence is inconclusive to recommend the use of Prosigna test to guide decisions for adjuvant endocrine and chemotherapy (EQ: Intermediate, RS: Moderate)

1.18. If a patient has node-positive breast cancer with more than 3 positive nodes, evidence on the clinical utility of routine use of Prosigna test to guide decisions for adjuvant endocrine and chemotherapy is insufficient to recommend its use (EQ: Insufficient, RS: Strong)

Extended Endocrine Therapy for ER Receptor-Positive HER2-Negative Breast Cancer

1.23. If a patient has node-negative breast cancer and has had 5 years of endocrine therapy without evidence of recurrence, there is insufficient evidence to use Oncotype DX, EndoPredict, Prosigna, Ki67, or IHC4 tests to guide decisions about extended endocrine therapy (EQ: Intermediate, RS: Moderate)

1.24. If a patient has node-negative or node-positive with 1-3 positive nodes breast cancer and has been treated with 5 years of primary endocrine therapy without evidence of recurrence, the clinician may offer BCI test to guide decisions about extended endocrine therapy with either tamoxifen, an AI or a sequence of tamoxifen followed by AI (EQ: Intermediate, RS: Moderate)

1.25. If a patient has node-positive breast cancer with more than 3 positive nodes and has been treated with 5 years of primary endocrine therapy without evidence of recurrence, there is insufficient evidence to use BCI test to guide decisions about extended endocrine therapy with either tamoxifen, an AI or a sequence of tamoxifen followed by AI (EQ: Intermediate, RS: Strong)

HER2-Positive Breast Cancer or Triple-Negative Breast Cancer

1.27. If a patient has HER2-positive breast cancer or TNBC, the clinician should not use multiparameter gene expression or protein assays (Oncotype DX, EndoPredict, MammaPrint, BCI, Prosigna, Ki67, or IHC4) to guide decisions for adjuvant endocrine and chemotherapy (EQ: Intermediate, RS: Strong)

The guidelines do not address the use of assays such as Oncotype DCIS or DCISionRT to guide decisions about radiation therapy in individuals with DCIS.

ASCO 2019 guidelines on the role of patient and disease factors in adjuvant systemic therapy decision-making for early-stage, operable breast cancer state:[79]

- Shared decision making between clinicians and patients is appropriate for adjuvant systemic therapy for breast cancer. For patients older than age 50 years and whose tumors have Oncotype DX recurrence scores less than 26, and for patients age 50 years or younger whose tumors have Oncotype DX recurrence scores less than 16, there is little to no benefit from chemotherapy. Clinicians may offer endocrine therapy alone for these patients. For patients age 50 years or younger with recurrence scores of 16 to 25, clinicians may offer chemoendocrine therapy. Patients with recurrence scores greater than 30 should be considered candidates for chemoendocrine therapy. Based on informal consensus, the Panel recommends that oncologists may offer chemoendocrine therapy to patients with Oncotype DX scores of 26 to 30.
- The MammaPrint assay could be used to guide decisions on withholding adjuvant systemic chemotherapy in patients with hormone receptor–positive lymph node–negative breast cancer and in select patients with lymph node–positive cancers. In both patients with node-positive and node-negative disease, evidence of clinical utility of the MammaPrint assay was only apparent in those determined to be at high clinical risk; the Panel thus did not recommend use of MammaPrint assay in patients determined to be at low clinical risk. Remaining recommendations from the 2016 ASCO guideline endorsement are unchanged.

American Society of Clinical Oncology/College of American Pathologists

In 2010, ASCO and the College of American Pathologists (CAP) issued recommendations on immunohistochemical testing for ER and PR, and issued recommendations in 2007[37, 80] (updated in 2014)[81] 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.

SUMMARY

ONCOTYPE DX®, BREAST CANCER INDEX™, AND ENDOPREDICT®

Oncotype DX® Breast Recurrence Score, Breast Cancer Index™, MammaPrint®, and EndoPredict® Assay in Node-Negative Patients and Patients with One to Three Positive Lymph Nodes

There is enough research to show that the Oncotype DX® Breast Recurrence Score, Breast Cancer Index™, MammaPrint®, and 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. In addition, the Breast Cancer Index™ may provide information to help make decisions regarding extended endocrine therapy. Clinical guidelines based on research consider these tests to be an option to help in making treatment decisions for individuals with breast cancer who do not have lymph node involvement, and those with 1-3 positive lymph nodes. Therefore, this testing may be considered medically necessary in patients when policy criteria are met.

Oncotype DX®, Breast Cancer Index™, MammaPrint®, and EndoPredict® Assay in Preliminary Biopsy Samples

There is not enough research to show that the use of the Oncotype DX® Breast Recurrence Score, Breast Cancer Index™, MammaPrint®, and EndoPredict® test on preliminary biopsy samples (prior to pathological evaluation) may improve health outcomes in breast cancer patients. The large studies that have validated these tests have primarily used surgical specimens. Full pathologic evaluation is important to determine the cellular and molecular features of a cancer, including lymph node status, prior to chemotherapy decision making. In addition, these tests have not been validated for use in making decisions for pre-surgical (neoadjuvant) therapy. Therefore, the use of these tests on preliminary biopsy samples is considered not medically necessary.

Oncotype DX®, Breast Cancer Index™, MammaPrint®, and EndoPredict® Assay in Patients with More than Three Positive Lymph Nodes

There is enough research to show that the use of the Oncotype DX® Breast Recurrence Score, Breast Cancer Index™, MammaPrint®, and EndoPredict® test may not improve health outcomes in breast cancer patients with more than three positive lymph nodes. For these patients, the risk of cancer recurrence without additional recommended therapy may be high. Therefore, testing in node-positive patients with more than three positive lymph nodes is considered not medically necessary.

Gene Expression Testing for DCIS

There is not enough research to show that gene expression tests for ductal carcinoma in situ (DCIS), including but not limited to the Oncotype DX® DCIS or DCISionRT, helps patients make treatment decisions that improve health outcomes. Clinical practice guidelines for breast cancer do not recommend this type of testing. Therefore, gene expression testing for DCIS 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.

Other Uses of Oncotype DX®, Breast Cancer Index™, MammaPrint®, or EndoPredict®

There is not enough research to show that using the Oncotype DX® Breast Recurrence Score, Breast Cancer Index™, or Endopredict® tests for purposes other than helping to decide whether to undergo adjuvant chemotherapy can improve survival and other health outcomes for patients with breast cancer. This includes using test results to make decisions about endocrine therapy, to predict response to specific chemotherapy regimens, or to evaluate response to treatments. In addition, there are no clinical guidelines based on research that recommend testing for these purposes. Therefore, the use of these tests for purposes other than helping to decide whether to undergo adjuvant chemotherapy is considered investigational.

BLUEPRINT® AND TARGETPRINT®

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

OTHER GENE EXPRESSION ASSAYS

There is not enough research to show that other gene expression assays for breast cancer, including the Molecular Grade Index (Aviara MGISM), Prosigna™, or BreastPRS™ tests can help breast cancer patients make treatment decisions that improve health outcomes. Therefore, these tests are considered investigational.

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CODES

Codes	Number	Description
CPT	0009U	Oncology (breast cancer), ERBB2 (HER2) copy number by FISH, tumor cells from formalin fixed paraffin embedded tissue isolated using image-based dielectrophoresis (DEP) sorting, reported as ERBB2 gene amplified or non-amplified
	0045U	Oncology (breast ductal carcinoma in situ), mRNA, gene expression profiling by real-time RT-PCR of 12 genes (7 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence score
	0153U	Oncology (breast), mRNA, gene expression profiling by next-generation sequencing of 101 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a triple negative breast cancer clinical subtype(s) with information on immune cell involvement
	0262U	Oncology (solid tumor), gene expression profiling by real-time RT-PCR of 7 gene pathways (ER, AR, PI3K, MAPK, HH, TGFB, Notch), formalin-fixed paraffinembedded (FFPE), algorithm reported as gene pathway activity score

Codes	Number	Description
	0295U	Oncology (breast ductal carcinoma in situ), protein expression profiling by immunohistochemistry of 7 proteins (COX2, FOXA1, HER2, Ki-67, p16, PR, SIAH2), with 4 clinicopathologic factors (size, age, margin status, palpability), utilizing formalin-fixed paraffin- embedded (FFPE) tissue, algorithm reported as a recurrence risk score
	81518	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 11 genes (7 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithms reported as percentage risk for metastatic recurrence and likelihood of benefit from extended endocrine therapy
	81519	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 21 genes, utilizing formalin-fixed paraffin embedded tissue, algorithm reported as recurrence score
	81520	Oncology (breast), MRNA gene expression profiling by hybrid capture of 58 genes (50 content and 8 housekeeping), utilizing formalin fixed paraffin-embedded tissue, algorithm reported as a recurrence risk score
	81521	Oncology (breast), MRNA, microarray gene expression profiling of 70 content genes and 465 housekeeping genes, utilizing fresh frozen or formalin-fixed paraffin-embedded tissue, algorithm reported as index related to risk of distant metastasis
	81522	Oncology (breast), mRNA, gene expression profiling by RT-PCR of 12 genes (8 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence risk score
	81523	Oncology, mRNA, next-generation sequencing gene expression profiling
HCPCS	S3854	Gene expression profiling panel for use in the management of breast cancer treatment

Date of Origin: *October 2004*