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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: February 1, 2019

Next Review: December 2019
Last Review: January 2019

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 cytotoxic chemotherapy. 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 Cancer Index^{SM}, 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)

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

II. Use of Oncotype DX®, Breast Cancer Index℠, MammaPrint®, or EndoPredict® to determine recurrence risk in patients with primary breast cancer who do not meet Criteria I. above is considered **not medically necessary**.

III. Use of Oncotype DX®, Breast Cancer Index℠, MammaPrint®, or EndoPredict® to determine patient risk in patients with primary breast cancer who meet Criteria I. above but who have already made the decision to undergo or forego chemotherapy is considered **not medically necessary**.

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 Cancer Index℠, MammaPrint®, or EndoPredict® for predicting response to specific chemotherapy or endocrine therapy regimens, determining HER2 status, or use in patients with other than stage I, II, or III breast cancer.

B. Use of other assays of genetic expression in breast tumor tissue, including but not limited to to BluePrint®, Mammostrat®, TargetPrint®, 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 IV.A.

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

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

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutations being tested
4. Relevant billing codes
5. Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing

6. Medical records related to this genetic test
    - History and physical exam
    - Conventional testing and outcomes
    - Conservative treatment provided, if any

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

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® (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^{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)
- 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 (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®.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[1] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term “variant” is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as “mutation.” Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

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^{SM}, and Prosigna™/PAM50 gene expression assay.[2] 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^{SM}, and Prosigna™ to determine recurrence risk in women with unilateral, hormone receptor-positive, lymph node-negative breast cancer who will receive hormonal therapy does not meet TEC criteria.

Earlier in 2014, the Agency for Healthcare Research and Quality (AHRQ) published a Technology Assessment of molecular pathology testing for the estimation of prognosis for common cancers, which included assessments of Oncotype DX® Breast and MammaPrint®.[3] AHRQ concluded that there was moderate evidence that Oncotype DX® Breast leads to changes in treatment decisions. The Technology Assessment stated:

> Although the decision changes were observed in both directions for individual patients, studies consistently showed an overall shift to less-intensive treatment recommendations as a result of using Oncotype DX® Breast, with fewer recommendations for chemotherapy (and therefore less exposure to potential harms of chemotherapy; but studies did not follow patients to actually report on harms or to assess the overall balance of clinical benefits and harms).

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

and imprecision.

ONCOTYPE DX® (GENOMIC HEALTH, INC.)

DESCRIPTION

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

Results from the Oncotype DX® gene expression profile are combined into a recurrence score (RS). Tissue sampling, rather than technical performance of the assay, is likely to be the greatest source of variability in results. The Oncotype DX® assay was validated in studies using archived tumor samples from subsets of patients enrolled in published RCTs of early breast cancer treatment. Patients enrolled in the trial arms, from which specimens were obtained, had primary, unilateral breast cancer with no history of prior cancer, and were treated with tamoxifen. Tumors were estrogen receptor positive, most were HER2-negative, and in the case of at least one study, multifocal tumors were excluded.[4]

ONCOTYPE DX® IN LYMPH NODE-NEGATIVE PATIENTS

TECHNOLOGY ASSESSMENTS

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

Other Studies in Lymph Node-Negative Patients

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

Sparano (2018) conducted a randomized controlled trial (RCT) (TAILORx) to evaluate risk of recurrence in women with midrange scores.[10] 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®.[5] 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.

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

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

- Those receiving aromatase inhibitor (AI)-based hormonal therapy instead of tamoxifen therapy. AI-based therapy would likely reduce recurrence rates for all RS risk groups. Thus, if a patient declined chemotherapy today on the basis of a low-risk RS (risk categories defined by outcomes with tamoxifen treatment), the even lower risk associated with AI treatment would not change that decision.
- Those receiving anthracycline-based chemotherapy instead of CMF. The type of chemotherapy does not change the interpretation of the Oncotype DX® risk estimate. Additionally, a recent meta-analysis indicates that anthracyclines do not improve disease-free or overall survival in women with early HER2-negative breast cancer[11], and therefore may not be prescribed in this population.
- Lymph nodes with micrometastases are not considered positive for purposes of treatment recommendations.[12] 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.[13,14] Studies documenting the low incidence (1% to 4%) and instability (lack of reproducibility) of the ER-negative/PR-positive subtype[15] and the reduction in reports of this subtype with improved assay techniques[16] suggest that this subtype may represent a false-negative result.

Several other nonrandomized studies reporting on the use of the 21-gene assay in lymph-node negative patients have been published[17,18], including a study by Sparano (2015) that assigned women with a recurrence score of 0 to 10 to receive endocrine therapy without chemotherapy.[19] 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.[20] 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.

A study by Toi (2010) confirmed the clinical validity of the 21-gene profile in a Japanese population of ER-positive, lymph node-negative patients, and had similar results for risk of distant recurrence in the three RS categories as the original validation studies.[21] Another study by Manounas (2010) investigated the association between RS and risk for locoregional recurrence (LRR), as opposed to distant recurrence, in patients from the two NSABP trials.[22] 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.

Several studies have been published regarding the impact of RS results on chemotherapy recommendations by medical oncologists.[23-31] 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.

Summary

For individuals who have early-stage node-negative invasive breast cancer considering adjuvant chemotherapy who receive gene expression profiling with Oncotype DX®, the evidence includes multiple prospective clinical trials and prospective-retrospective studies. Patients classified as low risk with Oncotype DX® have a low risk of recurrence in which avoidance of adjuvant chemotherapy is reasonable. These results have been demonstrated with stronger study designs for evaluating biomarkers. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

ONCOTYPE DX® IN LYMPH NODE-POSITIVE PATIENTS

Systematic Reviews

In a systematic review partly funded by Genomic Health, Brufsky (2014) [32] 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.

Nonrandomized Studies of Oncotype DX® in Lymph Node-Positive Patients

The following individual clinical studies were *not* included in the Brufsky (2014) review or the AHRQ Technology assessment described above.

Nitz (2017) conducted a phase 3 Plan B trial with a mixed population of women with node-negative and node-positive breast cancer.[33] 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 pN0/pN1 breast cancer and an RS of 11 or less. A total of 2,642 patients were included in the trial. Median age was 56 years, 59% were node-negative, 35% were pN1, and 6% were pN2-3. 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.

Gluz (2016) reported on a prospective study designed to evaluate outcomes of patients who are selected to avoid chemotherapy based on their RS score.[34] This study included patients with positive nodes. The sample size of patients with one to three positive nodes was 930, but the size of the sample followed for long term outcome is uncertain. Chemotherapy was deferred in patients who had RS < 12. The three-year disease-free survival for patients with one to three positive nodes who had RS < 12 was 97.9%. The three-year disease-free survival for patients with negative nodes was 98.6%. Although disease-free survival was similar between node-positive and node-negative patients at three years, the number of events was very small (eight total events) and follow-up is still early.

Ueno (2014)[35] conducted a small prospective study to evaluate the association between the Oncotype DX® RS and individual clinical response to neoadjuvant endocrine therapy in postmenopausal women with node-positive and node-negative breast cancer (n = 64). Study authors used archived tumor tissues from a previous study. Results of the assay and clinical response at baseline were compared with the same outcomes in patients with low assay result (< 18) and patients with high assay result (≥31). Inclusion criteria were as follows: 55 to 75 years of age; ER-POSITIVE and stage II or IIIa invasive breast cancer (T2-3, N0-2, M0). Treatment was exemestane (25 mg/day) for 16 weeks, with a possibility of an eight-week extension based on clinical response. The clinical response rate in patients with low RS (19/32, 59.4%) was significantly higher than patients with high RS (3/15, 20.0%) (p=0.015). Additional sub-analysis showed that patients with low RS had a significantly greater percentage of tumor reduction (nearly 32%) compared with patients with high RS who had an average tumor reduction of 12.5% (p=0.045). Rates of breast conserving surgery among the three groups were as follows: low RS (nearly 91%); intermediate RS (76.5%), and high RS

(nearly 47%). The odds ratio (OR) for breast conserving surgery between the high and low RS groups was 0.91 (95% CI 0.019 to 0.432, p=0.003). Study authors concluded that RS was predictive of the clinical response to neoadjuvant chemotherapy in postmenopausal women. This study was hampered by a few limitations, including its use of historical controls, small sample size, and lack of assessment of lymph node response following neoadjuvant endocrine treatment.

Markopoulos (2012) 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.[36] However, the study had a retrospective design and it is not clear whether all patients in this study had node-positive status.

Joh (2011) evaluated the impact of Oncotype DX® RS on chemotherapy recommendations and compared the estimated recurrence risk predicted by oncologists to RS.[28] 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.

Albain (2010) published retrospective analysis of the OncotypeDX® assay.[37] 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.

Summary

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

ONCOTYPE DX® IN PATIENTS WITH DUCTAL CARCINOMA IN SITU

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.[38]

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.[39] 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, 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).[40] Oncotype DX® DCIS Score was significantly associated with recurrence outcomes (hazard ratio [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.[41] 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.

Summary

These studies address the development of the Oncotype DX® DCIS Score and the clinical validity (statistical association of the test result with recurrence outcomes). Evidence for the clinical utility of Oncotype DX® DCIS is limited, and it is still uncertain if women are better categorized according to their recurrence risk using the Oncotype DX® DCIS Score compared with standard clinical indicators of risk. Although the DCIS score successfully stratifies patients into groups with different outcomes, it is unclear whether estimated recurrence risks for patients classified as low risk are low enough or estimated with sufficient precision to meaningfully affect the decision to have or forego radiotherapy.

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.[42] 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).[43]

Similarly, guidelines for HER2 testing specify IHC and/or FISH methods.[44] Although the HER2 component of the 21-gene assay has been shown to strongly correlate with FISH results,[45] the 21-gene assay should not be ordered to determine or confirm HER2.

MAMMAPRINT®

DESCRIPTION

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

TECHNOLOGY ASSESSMENTS

In the 2014 BCBSA TEC report, MammaPrint® did not meet TEC criteria in women with unilateral, hormone receptor-positive, lymph node-negative breast cancer who will receive hormonal therapy.[2]

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

OTHER STUDIES OF MAMMAPRINT®

A phase III study (MINDACT trial) published in 2016 enrolled 6,693 women with early-stage breast cancer and assessed their genomic risk using MammaPrint® and their clinical risk using a modified version of Adjuvant! Online for cancer recurrence.[46] Women with low risk by both indicators did not receive chemotherapy, women with high risk by both indicators did receive chemotherapy, and when the risk indicators did not agree, the use of chemotherapy was randomized, based on either the clinical or the genomic risk. Due to a change in MammaPrint® reagents, there was a temporary shift in the risk calculation that lasted nearly eight months. Because of this, 162 patients who had been identified as being at high genomic risk were

subsequently reclassified as having low genomic risk; 28 of these patients received chemotherapy prior to the correction, while the other 113 patients had their designations corrected. The primary endpoint for the study was a noninferiority outcome of 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®. Secondary analyses included outcome comparisons in patients in discordant risk groups between those who did and did not receive chemotherapy, outcome comparisons in all patients for whom chemotherapy was recommended by only one risk type, and calculation of the overall percentage of patients that would be assigned to chemotherapy based on either risk determination.

In this study, the median age of participants was 55 years (range 23 to 71), 79% had node-negative disease, 88.4% had ER/PR-positive disease, and 9.5% had HER2-positive disease. The clinical and genomic risks were discordant in 2,147 patients. There were 1,550 patients with high clinical risk and low genomic risk (as determined by MammaPrint®), and the five-year rate of survival without distant metastasis among those in this group who did not receive chemotherapy was 94.7% (95% CI 92.5 to 96.2), while this rate was 95.5% in those who did receive chemotherapy (approximate difference of 1.5%). The study was not adequately powered to reach statistical significance for this comparison. Based on these results, the authors concluded that chemotherapy could be avoided in the approximately 46% of high clinical risk breast cancers that are determined to be low genomic risk using MammaPrint®. The outcomes for participants at low clinical risk but high genomic risk who had chemotherapy were not meaningfully different than for those who did not have chemotherapy, so the information from the genomic risk test was not useful in those populations.

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.[47] 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.

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.[48] 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. Table 18 details survival rates for the initial low- and high-risk groups, and for the subgroup analysis that separated an ultralow-risk group.

Van 't Veer (2017) also published a study that used MammaPrint® data collected retrospectively from the Stockholm tamoxifen trial.[49] Both 10-year distant metastases-free survival (DMFS) and 20-year BCSS rates were calculated according to risk group and treatment group (tamoxifen vs. no treatment). Patients receiving tamoxifen experienced longer DMFS and BCSS in both the low- and high-risk groups compared with patients not receiving tamoxifen, with a 10-year DMFS for low-risk patients with tamoxifen of 93% (95% CI 88% to 96%) vs. 83% (95% CI 76% to 88%) for low-risk patients without tamoxifen.

A similar retrospective study was published by Groenendijk (2018), which used data from 1,916 patients in the Dutch Pathology Registry.[50] Clinical risk for 1,146 (58.9%) of the tumors was assessed retrospectively using Adjuvant! Online, and for 1,155 (59.4%) of the tumors using PREDICT. Although both MammaPrint® and Adjuvant! Online classified similar numbers of tumors as high and low risk (37.3% and 62.7% for Adjuvant! Online, and 38.0% and 62.0% for MammaPrint®, respectively), 52.6% (n = 428) of the clinically high-risk tumors were classified as low-risk by MammaPrint®.

Sapino (2014) published a validation study of MammaPrint® using formalin-fixed, paraffin-embedded (FFPE) tissue.[51] In a validation set of 221 tumor samples, concordance of FFPE and frozen tissue low- and high-risk classification was 91.5% (95% CI 86.9 to 94.5). Concordance of repeat analyses of the same tumor was 96%, and inter-laboratory reproducibility (i.e., between labs in the Netherlands and in California) was 96%.

The Microarray Prognostics in Breast Cancer (RASTER) study, published in 2013, was designed to assess feasibility of implementation and impact on treatment decisions of the MammaPrint® 70-gene signature, as well as recurrence outcomes.[52] The study followed 427 node-negative, early-stage breast cancer patients who had MammaPrint®, which was available to help direct post-surgery treatment decisions, and which was compared to Adjuvant! Online. All patients were aged 18 to 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 Adjuvant! Online occurred in 38% of the cases (161/427). Most discordant cases were MammaPrint® low-risk and Adjuvant! Online high-risk (124/427= 29%), whereas 37 cases (37/427=9%) had a high-risk MammaPrint® and a low-risk Adjuvant! Online estimation. Use of MammaPrint® reduced the proportion of high-risk patients as classified by Adjuvant! Online by 20% (87/427). The five-year distant recurrence-free interval probabilities were excellent for patients who were clinically high-risk but had a low-risk score with MammaPrint®, even in the absence of adjuvant systemic therapy.

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

Saghatchian (2013) evaluated MammaPrint® signatures of frozen tumor samples from patients who had four to nine positive lymph nodes.[53] Approximately half of patients were ER-positive, half were HER2-positive, and half had received adjuvant radiotherapy or chemotherapy. Seventy (40%) of 173 samples were classified as low risk by MammaPrint®, and 103 (60%) were classified as high risk. With median follow-up of eight years, five-year breast cancer-specific survival in the low and high-risk groups were 97% and 76%, respectively (log-rank test, p<0.01); five-year distant metastasis-free survival was 87% and 63%, respectively (log-rank test, p=0.004). Survival estimates were reported without 95% CIs; it is therefore not possible to assess the degree of overlap between risk groups.

Ahn (2013) investigated the use of MammaPrint® to further risk-stratify 82 ER-negative patients (56% lymph node-negative) who had Oncotype DX® intermediate risk scores.[54] Although MammaPrint® risk classification was significantly associated with 10-year overall survival in multivariate analysis (log-rank test, p=0.013), this result was confounded by receipt of adjuvant chemotherapy, which also was significantly associated with overall survival (log-rank test, p=0.024).

The 2012 I-SPY trial evaluated 237 patients with locally advanced disease (node-positive) by correlating imaging and MammaPrint® signatures with outcomes of pathologic complete response (pCR) and recurrence-free survival (RFS).[55] Despite having locally advanced disease, patients with 70-gene low-risk profiles tended not to respond to chemotherapy and to have good short-term RFS. However, there is only three years of follow-up, and the number of low risk patients was small.

Wittner (2008) 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.[56] 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 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.[57] However, the small study size (n=123) and small number of events precludes an adequate statistical analysis.[58] This study also updated results of the node-negative population from the validation study, reporting significantly different outcomes for good and poor gene signature prognosis groups, but estimates were very wide due to small numbers and a receiver operating characteristic (ROC) analysis also showed overlapping confidence intervals.[59]

Mook (2009) studied 241 node-positive patients with primarily ER-positive, HER2-negative tumors treated variably.[60] 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 one to three positive nodes from the validation study,[17] reporting 98% (95% CI 94 to 100%) 10-year breast cancer-specific survival for good prognosis signatures vs. 64% (95% CI 52 to 76%) for poor prognosis signatures; adjusted HR 3.63 (95% CI 0.88 to 14.96),

p=0.07). Based on these results, the ongoing MINDACT trial of MammaPrint® is being enlarged to include patients with one to three 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®.[61]

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

Other studies of MammaPrint® have been published, however the studies are generally small and/or retrospective or pooled re-analyses of subgroups from previously published retrospective studies.[62-69] In addition, several studies assessing the impact of MammaPrint® testing on treatment decision-making did not include survival or recurrence outcomes and are therefore considered uninformative for assessing clinical utility of MammaPrint®.[70,71]

ADDITIONAL APPLICATIONS OF MAMMAPRINT®

Drukker (2014) applied MammaPrint® to 1,053 tumor specimens from 1,848 patients enrolled in eight previous MammaPrint® studies in order to examine the ability of gene expression tests to provide risk information for locoregional recurrence.[72] The majority of patients had ER-positive, HER2-negative disease; approximately half of patients had positive axillary lymph nodes. The majority of patients received radiotherapy and did not receive adjuvant chemotherapy; approximately half received adjuvant endocrine therapy. At median follow-up of nine years, estimated 10-year locoregional recurrence risk was 13% (95% CI 10% to 16%) for 492 patients categorized as MammaPrint® high-risk versus 6% (95% CI 4% to 9%) for 561 MammaPrint® low-risk patients. This association was observed during the first five years after diagnosis, but not during years 5 to 10. Recurrence stratified by MammaPrint® risk class was not predictive of treatment response.

A study by Tsai (2017) assessed the impact on treatment decisions of using MammaPrint® for patients with an intermediate-risk result from the Oncotype DX®.[73] Among the 840 patients in this study that had an Oncotype DX® RS of 18 to 30, 374, (44.5%) were low-risk and 466 (55.5%) were high-risk according to MammaPrint®. The MammaPrint® results changed treatment recommendations for 279 of the patients: 108 (28.9%) of the low-risk patients had chemotherapy removed from the recommendations and 171 (36.7%) of the high-risk patients had chemotherapy added. Clinical outcomes were not available for analysis.

SUMMARY

The majority of MammaPrint® studies, including early validation studies, suffered from confounding due to heterogeneous patient samples. Subsequent pooled re-analyses of subpopulations controlled for one variable (e.g., nodal status), but confounding remained from other variables (e.g., treatment heterogeneity, ER status, HER2 status). It is therefore difficult to estimate outcomes for the patients of interest: ER-positive, HER-negative patients not receiving chemotherapy. Studies tended to be small and results had wide confidence intervals that could not rule out too much risk to consider withholding chemotherapy. Because the test result is not a continuous numerical result, patients cannot view their result within the spectrum of good prognosis results and adjust their preferences accordingly. However, the RASTER (Microarray Prognostics in Breast Cancer) study and the randomized MINDACT trial reported by by Cardoso (2016) represented improved study designs, and results of these studies

suggested that MammaPrint® may accurately re-classify early, node-negative breast cancer patients classified as high risk for recurrence by clinical and pathologic variables to low risk, such that chemotherapy may be avoided. Although the MINDACT trial has only five-year follow up data, it appears that most of the benefit of chemotherapy are seen within the first five years.[74] Thus the evidence is considered sufficient to demonstrate that the test may improve patient health outcomes.

BREAST CANCER INDEX^{SM} (BCI)

DESCRIPTION

The Breast Cancer Index^{SM} is a simultaneous assessment of the HOXB13:IL17BR (H/I) ratio and the MGI (Molecular Grade Index). The H/I ratio indicates estrogen-mediated signaling; MGI assesses tumor grade by measuring the expression of five cell-cycle genes and provides prognostic information in ER-positive patients regardless of nodal status. The 2014 TEC Assessment reviewed available studies for the original component assays.[2] There was insufficient evidence to determine whether the H/I ratio is better than conventional risk assessment tools in predicting recurrence. Ten-year recurrence estimates of patients classified as low risk were 17% to 25%, likely too high for most patients and physicians to consider forgoing chemotherapy. Studies of the combination Breast Cancer Index^{SM} (BCI) are reviewed below.

TECHNOLOGY ASSESSMENTS

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

OTHER STUDIES OF BREAST CANCER INDEX^{SM}

Schroeder (2017)[75] 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 multiinstitutional 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.[76] 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.

Sgroi (2013) examined 665 lymph node-negative, ER-positive, postmenopausal women receiving endocrine therapy but no chemotherapy in the ATAC trial. In this group, approximately 10% of samples were HER2+. Two versions of the Breast Cancer Index (BCI) score were generated in the study: the BCI-C, based on cubic combinations of the variables, and the BCI-L, based on linear combinations of the variables. The BCI-L, which is the model used in the development studies by Zhang et al. described above and represents the commercial version of the BCI, was more effective than the BCI-C at risk discrimination. The overall 10-year distant recurrence rates for the BCI-L low, intermediate, and high risk groups were 4.8% (95% CI 3.0% to 7.6%), 18.3% (95% CI 12.7% to 25.8%), and 29.0% (95% CI 21.1% to 39.1%), respectively. For patients in the low- and intermediate-risk groups, 10-year distant recurrence risks were similar, regardless of endocrine treatment (tamoxifen, anastrozole, or both).[77] 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).

Zhang (2013). evaluated a continuous risk model derived from the H/I ratio and MGI in tumor samples from the same RCT used by Jerevall (2011), described below (the Stockholm tamoxifen cohort; n = 317), along with additional samples from a multiinstitutional registry of ER-positive, lymph node-negative patients (n = 358), 32% of whom received adjuvant chemotherapy.[78] An optimized continuous recurrence risk model, the Breast Cancer Index^{SM} 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.

Jerevall (2011) 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.[79] The continuous model was also used to categorize patients into groups of low, intermediate, and high risk. This continuous predictor was tested in patients from the no adjuvant treatment arm (n=274) of the same clinical trial, with estimates of rates of distant recurrence or death at 10 years in the low, intermediate, and high-risk groups of 8.3% (95% CI 4.7% to 14.4%), 22.9% (95% CI 14.5% to 35.2%) and 28.5% (95% CI 17.9% to 43.6%), respectively. The estimates of breast cancer-specific death were 5.1% (95% CI 1.3% to 8.7%), 19.8% (95% CI 10.0% to 28.6%) and 28.8% (95% CI 15.3% to 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 (2011) evaluated tumor samples from 265 ER-positive, lymph node-negative, tamoxifen-treated patients from a single academic institution’s cancer research registry.[80] 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% to 10.9%), 12.1% (95% CI 2.7% to 21.5%), and 31.9% (95% CI 19.9% to 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%.

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 AVIARA MGI^{SM}

Ma (2008) evaluated MGI along with Aviara H/I^{SM} in a total of 733 patients.[81] 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.

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®

Stephen (2014) assessed the ability of Mammostrat® and IHC4 to provide information on the risk of early (within five years) or late (5 to 10 years) distant recurrence.[82] Tumor samples from two separate cohorts were analyzed: the Edinburgh Breast Conservation Series (n = 1103) with median follow-up of 12.9 years, and the Tamoxifen Exemestane Adjuvant Multinational (TEAM) trial (n = 3766) with median follow-up of 6.2 years. Patients had ER-positive disease and were treated with endocrine therapy without chemotherapy. Within the first five years after diagnosis, HRs comparing Mammostrat® high- with Mammostrat® low-risk patients were statistically significant only in the TEAM cohort, which had greater risk for relapse (greater mean tumor size, larger proportion of higher grade tumors, and greater mean number of positive lymph nodes) compared with the Edinburgh cohort. Measures of calibration (slope) and discrimination (R^2 statistic and index of discrimination) indicated that after five years (in the subset of patients who remained distant-recurrence free for at least five years, n = 3920 [81%]), there was no evidence of an association between Mammostrat® scores and time to distant recurrence.

Bartlett (2010) 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.[83]

Ross (2008) examined the same trial samples used for Oncotype DX® validation (NSABP B-14 and B-20 trials) and reported that among patients with early, node-negative breast cancer treated only with tamoxifen, those stratified by Mammostrat® into low-, moderate-, and high-risk groups had RFS estimates of 85%, 85%, and 73%, respectively.[84] Both low- and high-risk groups, but not moderate-risk groups, benefited significantly from chemotherapy treatment. A test for an interaction between chemotherapy and the risk group stratification was not significant (p=0.13).

Ring (2006) reported the development of the assay but provided no information on technical performance (analytic validity).[85] In an independent cohort, a multivariable model predicted 50%, 70%, and 87% five-year disease-free survival for patients classified as high, moderate, and low prognostic risk, respectively, by the test results (p=0.0008).

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

BREASTONCPX™

DESCRIPTION

The BreastOncPx™ test is a reverse transcriptase-polymerase chain reaction (RT-PCR) test performed on formalin-fixed, paraffin embedded tissue that measures the gene expression of 14 genes associated with key functions such as cell cycle control, apoptosis, and DNA recombination and repair. The results are combined into a metastasis score, which is reported to be associated with the risk of distant metastases in patients who are node-negative and estrogen-receptor positive.

STUDIES OF BREASTONCPX™

Tutt (2008) published information on the development and validation of the test.[86] No information on analytic validity was provided. Samples from untreated patients with early breast cancer were used to develop a gene signature that was completely prognostic for distant recurrence and not confounded by treatment prediction. 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 to 78%) for high risk patients and 96% (95% CI 90 to 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 to 8.44) and 1.97 (95% CI 1.28 to 3.04) for distant metastasis-free survival and overall survival, respectively. However, this difference in risk between groups was not maintained when the analysis was restricted to patients with tumors larger than 2 cm (p value for interaction 0.012).

ROC analysis of the continuous MS for distant metastasis and for death at 10 years, compared to Adjuvant!, resulted in slightly higher area under the curves (AUCs) for the MS in each case: 0.715 vs. 0.661 for distant metastases, and 0.693 vs. 0.655 for death. However, the MS was not added to Adjuvant! and was not compared to Adjuvant! alone. No reclassification analysis was conducted.

NEXCOURSE® BREAST IHC4

DESCRIPTION

NexCourse® Breast IHC4 evaluates the protein expression of ER/PR, HER2, and Ki-67 to provide a combined recurrence risk score. The assay technology uses quantitative image analysis to measure immunofluorescent signals, with results that can be combined in an algorithm to generate the recurrence risk score. The use of quantitative immunofluorescence is said to increase sensitivity, be more reproducible, and allow specific measurement of tumor cells.[87,88]

STUDIES OF NEXCOURSE® BREAST IHC4

In the Stephen study described above (see Mammostrat®), HRs comparing the interquartile range of the continuous IHC4 score were statistically significant in both the Edinburgh and TEAM cohorts within the first five years after diagnosis.[82] Measures of calibration and discrimination indicated that after five years, there was no evidence of an association between IHC4 scores and time to distant recurrence.

Cuzick (2011) 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® RS computed, and had adequate tissue for the IHC4 measurements.[89] Of these, 793 were node-negative and 59 were HER2-positive (but were not treated with trastuzumab). A prognostic model that combined the four immunohistochemical markers was created (IHC4). In a model combining either IHC4 or Oncotype DX® RS with classical prognostic variables, the IHC4 score was found to be similar to the Oncotype DX® RS, and little additional prognostic value was seen in the combined use of both scores. In a direct comparison, the IHC4 score was modestly correlated with the Oncotype DX® RS (r=0.72); the correlation was similar for node-negative patients (r=0.68). As an example, for a 1 to 2 cm, node-negative poorly differentiated tumor treated with anastrozole, nine-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 to 6.8).

Barton (2012) assessed the clinical utility of IHC4 plus clinicopathologic factors (IHC4 + C) by comparison with Adjuvant! Online and the Nottingham Prognostic Index (NPI)[90]. The study prospectively gathered clinicopathologic data for consecutively treated postmenopausal patients (n = 101 evaluable) with hormone receptor-positive, HER2-negative, lymph node-negative or positive with one or two nodes, resected early breast cancer. Of 59 patients classified as intermediate-risk group by the NPI, IHC4 reclassified 24 to low risk and 13 to high risk. IHC4 reclassified 13 of 32 Adjuvant! high-risk patients to intermediate risk, and three of 32 to low risk. In addition, 15 of 26 Adjuvant! intermediate-risk patients were reclassified to low risk. No Adjuvant! low-risk patients were reclassified high risk.

PROSIGNA™/ PAM50 BREAST CANCER INTRINSIC SUBTYPE CLASSIFIER

DESCRIPTION

PAM50 Breast Cancer Intrinsic Classifier, a qRT-PCR test based on a panel of 50 genes, was developed to identify the breast cancer intrinsic subtypes known as luminal A, luminal B, HER2-enriched, and basal-like, and to generate risk-of-relapse scores in node-negative patients who had not had systemic treatment for their cancer. Prosigna™ evolved from the PAM50 test and uses NanoString's nCounter platform[91] in place of qRT-PCR to assay 46 genes instead of the original 50.

TECHNOLOGY ASSESSMENT

The 2014 TEC Assessment reviewed development and validation studies of the PAM50 intrinsic subtype classifier and Prosigna™;[2] these studies are reviewed below. Only two studies of the marketed Prosigna™ test were identified, one of which reported analytic validity. A third study performed the commercial assay on 46 of the PAM50 genes, excluding one HER2-associated gene (*GRB7*) and three proliferation-associated genes (*BIRC5* [also called *Survivin*], *MYBL2*, and *CCNB1*], that are given special weighting to generate the Prosigna™ recurrence of recurrence (ROR) score. These and other studies published after the 2014 TEC Assessment are reviewed below.

STUDIES OF PROSIGNA™/PAM50 FOR RECURRENCE RISK

Two studies published in 2015 presented combined analyses of pretreatment FFPE tumor specimens from ABCSG-8 and ATAC trial monotherapy arms (TransATAC).[92,93] 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).[92] 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.[93] 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.[89] 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),[94] 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 recurrence-free survival (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.

Ohnstad (2017) evaluated the prognostic value of PAM50–determined intrinsic subtypes and ROR scores in 653 samples from participants in the Oslo1 study.[95] Samples used for this study were from early, hormone receptor-positive, HER2-negative, lymph node-negative breast cancers not treated with chemotherapy. There were 231 patients that had no adjuvant treatment, and 53.7% of these had a low ROR. The 15-year BCSS among these low-ROR patients was 96.3%, which was significantly higher than those with intermediate ROR scores (p=0.005). There was no difference seen between low and intermediate ROR scores for patients that received tamoxifen only.

Liu (2015) assessed the prognostic and predictive value of PAM50 using 1,094 breast tumor samples from the National Cancer Institute of Canada's MA.21 trial.[96] MA.21 was an international phase 3 trial that compared taxane and non-taxane chemotherapy in 2,104 premenopausal or postmenopausal women 60 years of age or younger with node-positive or high-risk node-negative breast cancer. Patients were stratified by type of surgery (partial or total mastectomy), number of positive axillary lymph nodes, and ER status. Approximately 60% of patients were ER-positive, and approximately 60% received adjuvant endocrine therapy. PAM50 subtypes and ROR scores were determined using the nCounter Analysis system. Of all samples tested (52% of patients randomized), 3%, 18%, and 79% were classified as ROR low-, intermediate-, and high-risk, respectively. In multivariate analysis, ROR score on a continuous scale was statistically associated with RFS, but categorical ROR was associated with neither RFS nor survival by treatment group (i.e., neither prognostic nor predictive). Intrinsic subtypes were associated with RFS but were not predictive of treatment outcomes. The authors stated:[96]

> "The characteristics of the study population of MA.21, which includes more high-risk breast cancer patients, are different from those used for the development and validation of the NanoString PAM50 ROR score classification. Thus, we suggest that researchers need to be cautious when applying the ROR risk classification in different study populations. Compared with ROR score, intrinsic subtype is expected to be more reliable for predicting clinical outcome and response to therapies in different breast

cancer populations as it is based on the fundamental biology of breast cancer, whereas the ROR algorithm was optimized against outcome in a specific population."

Cheang (2012) determined PAM50 intrinsic subtypes for samples from a clinical trial that randomized premenopausal women with node-positive breast cancer to two different regimens of chemotherapy. The PAM50 intrinsic subtype for 476 tumors was correlated to RFS ($p=0.0005$) and overall survival ($p<0.0001$).[97] The HER2-enriched subgroup (22%) showed the greatest benefit from cyclophosphamide-epirubicin-fluorouracil (CEF) versus cyclophosphamide-methotrexate-fluorouracil (CMF), with absolute five-year RFS and Overall survival differences exceeding 20%. There was a less than 2% difference for non–HER2-enriched tumors (interaction test $p=0.03$ for RFS and 0.03 for survival). Within clinically defined HER2-positive tumors, 79% (72 of 91) were classified as the HER2-enriched subtype by gene expression, and this subset was associated with better response to CEF versus CMF (62% vs. 22%, $p=0.0006$). There was no significant difference in benefit from CEF versus CMF in basal-like tumors.

The following studies were included in the 2014 TEC Assessment:

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

In a study that supported FDA clearance of Prosigna™, Gnant (2014) evaluated tumor samples from 1047 lymph node-negative patients who participated in the Austrian Breast and Colorectal Cancer Study Group's trial 8 (ABCSG-8); this represented 28% of the original trial sample.[94] 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

Filipits (2014). subsequently studied 919 patients who survived the first five years after treatment without recurrence.[99] Fifteen-year late-distant recurrence-free survival (i.e., years 5-15) was 98%, 90%, and 86% in ROR low-, intermediate-, and high-risk groups, respectively.

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.[100] 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.

Sestak (2013) reported on the prognostic ability of PAM50 ROR score in 940 (16%) of 5880 patients from the ATAC trial.[101] 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.

In an earlier study, Nielsen (2010) compared the PAM50 classifier with standard clinicopathologic factors as represented by Adjuvant! Online and with models based on immunohistochemistry for biomarkers of intrinsic subtypes.[102] The study used samples from patients diagnosed between 1986 and 1992 with ER-positive breast cancer, either higher-risk (e.g., with lymphovascular invasion) node-negative or node-positive disease, and treated with five years of tamoxifen but no adjuvant chemotherapy. In the node-negative population, Adjuvant! Online was inferior to all other biomarker models for predicting recurrence and disease-specific survival. A model including the PAM50 risk of recurrence gene expression signature that also incorporated the influence of proliferation and tumor size identified patients with a greater than 95% chance of remaining alive and disease-free beyond 10 years. A slightly different gene expression model best fit the node-positive population, but did not identify a sufficiently low-risk population wherein adjuvant hormone therapy would likely be considered sufficient. Because the cohort used to generate the models evaluated in this study was biased toward higher-risk early breast cancers, this finding is likely not generalizable to other populations. In addition, the authors did not clearly identify a final model for clinical use.

The initial development of the PAM50 Breast Cancer Intrinsic Classifier was reported by Parker (2009).[103] In an independent test set, the test using three categories of risk (low, intermediate, and high) was significantly prognostic (log-rank p=0.0002).

OTHER STUDIES OF PROSIGNA™/PAM50

Researchers have also evaluated other uses of the PAM50 in smaller studies. For example, Kimbung (2018) found that post-chemotherapy changes in PAM50 were correlated with event-free survival in a study of 150 patients with HER2-negative, locally advanced breast cancers,[104] and Laenkholm (2018) evaluated the use of the PAM50 in 89 breast cancer patients with special histological subtypes.[105] A study by Laurberg (2018) evaluated whether the PAM50 intrinsic subtypes could be used to predict benefit from adjuvant radiotherapy in

two postmastectomy trials, and found all patients, including those with Luminal A tumors, had a significantly reduced incidence of loco-regional recurrence after radiotherapy.[106] Another study found that PAM50 results from lymph node metastases instead of primary tumors were correlated with BCSS.[107]

Sánchez-Muñoz (2017) evaluated the use of the PAM50 in male patients with breast cancer.[108] A research version of the PAM50 was applied to 67 samples from for pathology laboratories in Spain, which identified 30% as luminal A, 60% as luminal B, and 10% as HER2 enriched. IHC testing identified 44% as luminal A, 51% as luminal B, 4% as triple-negative, and 1% as HER2 enriched. The authors reported that individuals that were HER2-negative by IHC but HER2-enriched according to the PAM50 had worse outcomes than the luminal subtypes. A similar study was win 607 patients was reported by Kim (2018).[109]

Hequet (2017)[110] and Martin (2015)[111] 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™.

CONCLUSION

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®

DESCRIPTION

Gene expression patterns have led to the identification of molecular subtypes of breast cancer, which have different prognoses and responses to treatment regimens. These molecular subtypes are largely distinguished by differential expression of 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® offers a quantitative assessment of ER, PR and HER2 overexpression in breast cancer. Both BluePrint® and TargetPrint® are intended for use with MammaPrint®.

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

STUDIES OF BLUEPRINT® AND TARGETPRINT®

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.[112] 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.[113] 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.

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.[114] 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.

Viale (2014) reported concordance between TargetPrint® and IHC testing for ER and PR and FISH for HER2 in the first 800 patients enrolled in the pilot phase of the MINDACT MammaPrint® trial.[115] For ER, positive and negative percent agreement between TargetPrint® and central testing were 98% and 96%, respectively; positive (PPV) and negative predictive value (NPV) were 99% and 87%, respectively. For PR, positive and negative percent agreement were 83% and 91%, respectively; PPV and NPV were 97% and 59%, respectively. For HER2, positive and negative percent agreement were 75% and 99%, respectively; PPV and NPV were 91% and 97%, respectively.

Nguyen (2012) compared molecular subtyping with BluePrint®, MammaPrint® and TargetPrint® to locally assess clinical subtyping using IHC and FISH.[116] 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 analyses were performed on 132 samples. The concordance between BluePrint® and IHC and FISH testing was 94% for both the basal-type and luminal-type subgroups, and 95% for the HER2-type. The concordance of BluePrint® with subtyping using mRNA readout (TargetPrint®) was 98% for the basal-type, 96% for the luminal-type, and 97% for the HER2 type. The authors concluded that implementation of these multigene assays may improve the clinical management of breast cancer patients by including substratification rather than tumor grade alone.

The BluePrint® molecular subtyping profile was developed using 200 breast cancer specimens that had concordant ER, PR and HER2 protein levels by immunohistochemistry and TargetPrint® mRNA readout.[117] 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.

BREASTPRS™

DESCRIPTION

BreastPRS™ is a gene expression assay that analyzes 200 genes in its algorithm, and was validated from a meta-analysis of publically available genomic datasets.[118] BreastPRS™ is a binary assay which stratifies patients into low- and high-risk groups.[119]

STUDIES OF BREASTPRS™

D'Alfonso (2013) sought to translate a previously published validation study of BreastPRS™, using fresh-frozen tissue, to FFPE tumor samples.[119] The authors compared the BreastPRS prognostic index to the Oncotype DX® assay and correlated recurrence scores with clinicopathologic features. They also used publically available whole genome profiles from a series of untreated ER-POSITIVE, node-negative patients to investigate the ability of BreastPRS™ to reclassify Oncotype DX® intermediate-risk patients into high- versus low-risk categories with clinically significant differences in outcome. A linear relationship of the BreastPRS™ prognostic score was observed between fresh-frozen and FFPE formats. BreastPRS™ recurrence scores were compared with Oncotype DX® recurrence scores from 246 patients with invasive breast carcinoma and known Oncotype DX® results. Using this series, a 120-gene Oncotype DX® approximation algorithm to predict Oncotype DX® risk groups was then applied to a series of untreated, ER-positive, node-negative patients from previously published studies with known clinical outcomes. Of the 30 high-risk Oncotype DX® cases, 27 (90%) were classified as high-risk by BreastPRS™, and 95 low-risk Oncotype DX® cases (76%) were classified as low-risk by BreastPRS™. The correlation of recurrence score and risk group between Oncotype DX® and BreastPRS™ was statistically significant ($p<0.0001$). Fifty-nine of 260 (23%) patients from four previously published studies were classified as intermediate-risk when the 120-gene Oncotype DX® approximation algorithm was applied. BreastPRS™ reclassified the 59 patients into binary risk groups (high- vs. low-risk), with 23 (39%) patients classified as low-risk and 36 (61%) as high-risk (HR 3.64, 95% CI 1.40 to 9.50, $p=0.029$). At 10 years from diagnosis, the low-risk group had a 90% RFS rate compared to 60% for the high-risk group. The authors concluded that the BreastPRS™ recurrence score is comparable with Oncotype DX® and can reclassify Oncotype DX® intermediate-risk patients into two groups with significant differences in RFS. The authors noted further studies are necessary to validate these findings.

ENDOPREDICT®

DESCRIPTION

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

STUDIES OF ENDOPREDICT®

Filipits (2011) reported on the validation of EndoPredict® using tumor samples from women receiving endocrine treatment in the ABCSG-6 and ABCSG-8 trials.[120] 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.

Buus (2016) evaluated EndoPredict® as a prognostic indicator for breast cancer recurrence in women treated endocrine therapy.[121] 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.

Bertucci (2014) evaluated 553 ER-positive/HER2-negative breast cancers treated with anthracycline-based neoadjuvant chemotherapy.[122] Fifty-one percent of samples were classified as EndoPredict® low-risk with a pCR rate of 7%; 49% of samples were classified as EndoPredict® high-risk with a pCR rate of 17%. Estimated five-year disease-free survival was 88% (95% CI 81 to 95) in the EndoPredict® low-risk group and 73% (95% CI 63 to 85) in the EndoPredict® high-risk group.

Martin (2014) assessed tumor samples from 566 ER-positive, HER2-negative patients who participated in the GEICAM 9906 RCT.[123] 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.

Dubsky (2013) examined predictive ability of EP and EPclin for early (within five years) and late (more than five years post-diagnosis) disease recurrence.[124] 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).

ADDITIONAL APPLICATIONS OF ENDOPREDICT®

Fitzal (2015) evaluated local recurrence using EndoPredict® in breast tumor samples from 1324 patients who had participated in the ABCSG-8 trial (29% of enrolled patients), which compared adjuvant endocrine therapy regimens.[125] The majority of patients had node-negative, ER-positive disease and received breast-conserving surgery and radiotherapy; approximately half of patients received adjuvant endocrine therapy. At median follow-up of six years, Kaplan-Meier estimated 10-year risk of local RFS was 96% (91% reported in the article abstract) among 683 patients classified by EndoPredict® as high risk versus 99% among 641 patients classified by EndoPredict® as low-risk. EndoPredict® risk groups were not associated with treatment outcomes.

Additional smaller, nonrandomized studies have evaluated the use of EPclin to predict chemotherapy response,[126] and compared EPclin to a computational risk prediction algorithm.[127]

TEST COMPARISON STUDIES

A systematic review by Blok (2018) assessed the clinical utility of gene expression profiles for breast cancer in Europe. Endopredict®, MammaPrint®, Oncotype DX®, and Prosigna™/PAM50 were evaluated in the review, which included 147 articles.[128] Level IA clinical evidence was found for MammaPrint® and Oncotype DX®. Oncotype DX® was the only assay that had demonstrated predictive value, with clinical utility studies showing a greater reduction of chemotherapy with this test. The authors noted that while EndoPredict® and Prosigna™/PAM50 demonstrated similar prognostic capacities, there were fewer clinical utility studies and no level IA trial evidence for these assays. A systematic review of these four assays by Chang (2017), which included 24 articles, came to similar conclusions.[129]

Sestak et al (2018) compared Breast Cancer Index®, Oncotype DX®, Prosigna®, and Endopredict® using samples from the TransATAC RCT.[130] 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.[131] 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%).

Research versions of the 70-gene, cell-cycle score, Genomic Grade Index, PAM50, and RS were compared to Ki67 alone or in combination with ER, PR, and HER2 (IHC subtypes), in a study be Lundberg (2017).[132] This study used data from two Swedish cohorts with 379 and 209 participants, and median follow-up times of 12.4 and 12.5 years. The authors reported that the RS and PAM50 provided more prognostic data than the IHC subtypes in all participants, but that the IHC added prognostic information to all molecular profiles except PAM50.

Sgroi (2013) compared the Breast Cancer Index^{SM} and Oncotype DX® in 665 lymph node-negative women receiving endocrine therapy but not chemotherapy in the ATAC trial.[77] 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^{SM} was a better predictor of risk: 5% of Breast Cancer Index^{SM} low-risk patients had distant recurrence compared with 9% of Oncotype DX® low-risk patients, and 22% of Breast Cancer Index^{SM} 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)[133] examined cross-stratification between the Breast Cancer Index^{SM} 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.[100] 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.

Hornberger (2012) 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.[134] There were 56 articles that published original evidence addressing the 21-gene recurrence score (Oncotype DX®, n = 31), 70-gene signature (MammaPrint®, n = 14), Adjuvant! Online (n = 12), five-antibody immunohistochemistry panel (Mammostrat®, n = 3), and 14-gene signature (BreastOncPx™, n = 1). The results of the review found that Oncotype DX® recurrence score satisfied level I evidence for estimating distant recurrence risk (DRR), OS, and response to adjuvant chemotherapy, and level II evidence for estimating local recurrence risk. Mammostrat® and MammaPrint® satisfied level II evidence for estimating DRR and OS. Adjuvant! Online satisfied level 2 evidence for estimating DRR, OS, and chemotherapy response. BreastOncPx™ satisfied level 3 evidence for predicting DRR and OS. Ten studies reported changes in clinical practice patterns using the 21-gene recurrence score. Overall, the 21-gene recurrence score was associated with change in treatment recommendations and/or decisions in 20.6% to 74.0% of cases.

Varga (2013) analyzed the EndoPredict® test in 34 hormone positive, invasive breast cancer cases and compared the EP scores with the Oncotype DX® RS obtained from the same cancer samples.[135] EP classified 11 patients as low-risk and 23 patients as high-risk, whereas the RS Score defined 15 patients as low-risk, 10 patients as intermediate-risk in and nine patients as high-risk. There were major discrepancies in six of 34 cases (18%), with low-risk RS classified as high-risk by EP in six cases. When the RS intermediate and high-risk groups were combined, the concordance between both tests was 76%. The clinical relevance of these discrepant test results with respect to outcome is unknown.

Similarly, the study by Buus (2016) described earlier, compared EndoPredict® with Oncotype DX® RS in hormone receptor-positive, HER2-negative tumor samples from the TransATAC study.[121] 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.

Fan (2006) used five gene expression classifiers to evaluate a single set of samples from 295 women with stage 1 or 2 breast cancer, variable node involvement, and variable endocrine or chemotherapy treatment.[136] The classifiers included the 21-gene RS, 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 RS 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 (2005) compared the 21-gene Oncotype DX® RS, the 70-gene signature (MammaPrint®), and the H/I Ratio in 153 patients with ER-positive breast cancer treated with adjuvant tamoxifen.[137] Of these patients, 38% were node-positive and 63% were additionally treated with chemotherapy. Distant metastasis-free survival for the RS was 98% for low-risk patients versus 81% for intermediate-risk versus 69% for high-risk; for the 70-gene signature the estimates were 95% good prognosis versus 66% poor prognosis; and for the H/I Ratio, 86% favorable versus 70% unfavorable. There was a good correlation between the 21-gene RS 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 RS 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 papers from 2012 compared the Oncotype DX® and other gene expression profiles. Kelly (2012) evaluated Oncotype DX® and PAM50 in 108 cases and found good agreement between the two assays for high- and low-prognostic risk assignment, but PAM50 assigned about half of Oncotype DX® intermediate-risk patients to the PAM50 luminal A (low-risk) category.[138] Prat (2012) 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 to 0.79; $p<0.0001$ for each comparison).[27]

PRACTICE GUIDELINE SUMMARY

National Comprehensive Cancer Network®[12]

NCCN guidelines for breast cancer (version 3.2018) recommend that the Oncotype DX® assay be strongly considered in node-negative, HR-positive, HER2-negative disease when the tumor is >0.5 cm, stage pT1, pT2, or pT3, and of ductal, lobular, mixed or metaplastic histology (category 2A). Regarding node-positive, HR-positive, HER2-negative disease, the guidelines state that, "the 21-gene RT-PCR assay recurrence score can be considered in select patients with 1 to 3 involved ipsilateral ALNs to guide the addition of combination chemotherapy to standard hormone therapy." 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.

Currently NCCN does not address the use of the Molecular Grade Index, Mammostrat®, BreastOncPx™, IHC4, BluePrint®, TargetPrint®, or BreastPRS™ assays.

American Society of Clinical Oncology (ASCO)[139]

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

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

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

The ASCO guidelines state that the Breast Cancer Index℠, EndoPredict®, and PAM50 may be used in women with ER/PgR-positive, HER2-negative, node-negative breast cancer. For the Breast Cancer Index℠ and EndoPredict®, the strength of these recommendations is considered moderate and based on intermediate quality evidence by the guideline authors. The recommendation for the PAM50 is considered strong and based on high-quality evidence, however it is based on three studies. All three studies were industry sponsored prospective analyses on retrospectively collected cohorts and focused on the clinical validity and the potential for the test to impact treatment decisions, but did not directly demonstrate clinical utility.

Currently, ASCO does not address the use of the Molecular Grade Index (Aviara MGI℠) as an option when evaluating breast cancer patients for risk of recurrence, or the use of BreastOncPx™, BreastPRS™,BluePrint® and TargetPrint® as an option when evaluating breast cancer patients for risk of recurrence.

The guidelines recommend against the use of Mammostrat®, stating that the group of patients considered low-risk by the assay had 10-year recurrence risks that were low, and the use of IHC4, stating that the test is not sufficiently reproducible, despite evidence of clinical utility.

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[44,140] (updated in 2014)[141] 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.

U.S. Preventative Services Task Force (USPSTF)[142]

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

SUMMARY

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

Oncotype DX®, Breast Cancer Index℠, MammaPrint®, and EndoPredict® Assay in Node-Negative Patients

There is enough research to show that the Oncotype DX®, 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. Clinical guidelines based on research consider this test to be an option to help in making treatment decisions for individuals with breast cancer who do not have lymph node involvement. Therefore, this testing may be considered medically necessary in lymph node-negative patients when policy criteria are met.

Oncotype DX®, Breast Cancer Index℠, MammaPrint®, and EndoPredict® Assay in Node-Positive Patients

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

Oncotype DX® Assay in DCIS Patients

There is not enough research to show that using Oncotype DX® DCIS helps patients with ductal carcinoma in situ (DCIS) make treatment decisions that improve health outcomes. Therefore, Oncotype DX® 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 IndexSM, MammaPrint®, or EndoPredict®

There is not enough research to show that using the Oncotype DX®, 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.

MOLECULAR GRADE INDEX (AVIARA MGISM), MAMMOSTRAT®, BREASTONCPX™, PROSIGNA TM, NEXCOURSE® BREAST IHC4, BREASTPRS™, OTHERS

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

REFERENCES

1. den Dunnen, JT, Dalgleish, R, Maglott, DR, et al. HGVS Recommendations for the Description of Sequence Variants: 2016 Update. *Human mutation*. 2016 Jun;37(6):564-9. PMID: 26931183
2. TEC Assessment 2014. "Gene expression profiling in women with lymph node negative breast cancer to select adjuvant chemotherapy." BlueCross BlueShield Association Technology Evaluation Center, Vol. 29, Tab 3.
3. Meleth, S, Reeder-Hayes, K, Ashok, M, et al. Technology Assessment of Molecular Pathology Testing for the Estimation of Prognosis for Common Cancers. 2014. PMID: 25905152
4. Paik, S, Tang, G, Shak, S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol*. 2006 Aug 10;24(23):3726-34. PMID: 16720680
5. Paik, S, Shak, S, Tang, G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*. 2004 Dec 30;351(27):2817-26. PMID: 15591335
6. Paik, S, Shak, S, Tang, G, et al. Risk classification of breast cancer patients by the Recurrence Score assay: comparison to guidelines based on patient age, tumor size, and tumor grade. *Breast Cancer Res Treat*. 2004b;88(Suppl 1):A104 [Abstract]. PMID: No PMID Entry
7. Bryant J. Toward a more rational selection of tailored adjuvant therapy data from the National Surgical Adjuvant Breast and Bowel Project. 2005 St. Gallen Breast Cancer Symposium. [Complete slide presentation via Genomic Health].
8. Habel, LA, Shak, S, Jacobs, MK, et al. A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients. *Breast Cancer Res*. 2006;8(3):R25. PMID: 16737553
9. TEC Assessment 2005. "Gene expression profiling for managing breast cancer treatment." BlueCross BlueShield Association Technology Evaluation Center, Vol. 20, Tab 3.
10. Sparano, JA, Gray, RJ, Makower, DF, et al. Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med*. 2018 Jul 12;379(2):111-21. PMID: 29860917

11. Gennari, A, Sormani, MP, Pronzato, P, et al. HER2 status and efficacy of adjuvant anthracyclines in early breast cancer: a pooled analysis of randomized trials. *J Natl Cancer Inst*. 2008 Jan 2;100(1):14-20. PMID: 18159072
12. National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology™. Breast Cancer. v.3.2018. [cited 01/03/2019]; Available from: http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf
13. Dowsett M, on Behalf of the ATAC Trialists Group. Analysis of time to recurrence in the ATAC (arimidex, tamoxifen, alone or in combination) trial according to estrogen receptor and progesterone receptor status. 26th Annual San Antonio Breast Cancer Symposium, 2003.
14. Dowsett, M, Houghton, J, Iden, C, et al. Benefit from adjuvant tamoxifen therapy in primary breast cancer patients according oestrogen receptor, progesterone receptor, EGF receptor and HER2 status. *Ann Oncol*. 2006 May;17(5):818-26. PMID: 16497822
15. Hefti, MM, Hu, R, Knoblauch, NW, et al. Estrogen receptor negative/progesterone receptor positive breast cancer is not a reproducible subtype. *Breast Cancer Res*. 2013;15(4):R68. PMID: 23971947
16. Davies, C, Godwin, J, Gray, R, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet*. 2011 Aug 27;378(9793):771-84. PMID: 21802721
17. Tzeng, JP, Mayer, D, Richman, AR, et al. Women's experiences with genomic testing for breast cancer recurrence risk. *Cancer*. 2010 Apr 15;116(8):1992-2000. PMID: 20213682
18. Tang, G, Shak, S, Paik, S, et al. Comparison of the prognostic and predictive utilities of the 21-gene Recurrence Score assay and Adjuvant! for women with node-negative, ER-positive breast cancer: results from NSABP B-14 and NSABP B-20. *Breast Cancer Res Treat*. 2011 May;127(1):133-42. PMID: 21221771
19. Sparano, JA, Gray, RJ, Makower, DF, et al. Prospective Validation of a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med*. 2015 Nov 19;373(21):2005-14. PMID: 26412349
20. Kizy, S, Huang, JL, Marmor, S, Tuttle, TM, Hui, JYC. Impact of the 21-gene recurrence score on outcome in patients with invasive lobular carcinoma of the breast. *Breast Cancer Res Treat*. 2017 Oct;165(3):757-63. PMID: 28647915
21. Toi, M, Iwata, H, Yamanaka, T, et al. Clinical significance of the 21-gene signature (Oncotype DX) in hormone receptor-positive early stage primary breast cancer in the Japanese population. *Cancer*. 2010 Jul 1;116(13):3112-8. PMID: 20564629
22. Mamounas, EP, Tang, G, Fisher, B, et al. Association between the 21-gene recurrence score assay and risk of locoregional recurrence in node-negative, estrogen receptor-positive breast cancer: results from NSABP B-14 and NSABP B-20. *J Clin Oncol*. 2010 Apr 1;28(10):1677-83. PMID: 20065188
23. Lo, SS, Mumby, PB, Norton, J, et al. Prospective multicenter study of the impact of the 21-gene recurrence score assay on medical oncologist and patient adjuvant breast cancer treatment selection. *J Clin Oncol*. 2010 Apr 1;28(10):1671-6. PMID: 20065191
24. Henry, LR, Stojadinovic, A, Swain, SM, Prindiville, S, Cordes, R, Soballe, PW. The influence of a gene expression profile on breast cancer decisions. *J Surg Oncol*. 2009 May 1;99(6):319-23. PMID: 19204954
25. Klang, SH, Hammerman, A, Liebermann, N, Efrat, N, Doberne, J, Hornberger, J. Economic implications of 21-gene breast cancer risk assay from the perspective of an Israeli-managed health-care organization. *Value Health*. 2010 Jun-Jul;13(4):381-7. PMID: 20412544

26. Ademuyiwa, FO, Miller, A, O'Connor, T, et al. The effects of oncotype DX recurrence scores on chemotherapy utilization in a multi-institutional breast cancer cohort. *Breast Cancer Res Treat*. 2011 Apr;126(3):797-802. PMID: 21197567
27. Prat, A, Parker, JS, Fan, C, et al. Concordance among gene expression-based predictors for ER-positive breast cancer treated with adjuvant tamoxifen. *Ann Oncol*. 2012 Nov;23(11):2866-73. PMID: 22532584
28. Joh, JE, Esposito, NN, Kiluk, JV, et al. The effect of Oncotype DX recurrence score on treatment recommendations for patients with estrogen receptor-positive early stage breast cancer and correlation with estimation of recurrence risk by breast cancer specialists. *Oncologist*. 2011;16(11):1520-6. PMID: 22016474
29. Hassett, MJ, Silver, SM, Hughes, ME, et al. Adoption of gene expression profile testing and association with use of chemotherapy among women with breast cancer. *J Clin Oncol*. 2012 Jun 20;30(18):2218-26. PMID: 22585699
30. Carlson, JJ, Roth, JA. The impact of the Oncotype Dx breast cancer assay in clinical practice: a systematic review and meta-analysis. *Breast Cancer Res Treat*. 2013 Aug;141(1):13-22. PMID: 23974828
31. Rath, MG, Uhlmann, L, Fiedler, M, et al. Oncotype DX((R)) in breast cancer patients: clinical experience, outcome and follow-up-a case-control study. *Arch Gynecol Obstet*. 2017 Dec 13. PMID: 29236174
32. Brufsky, AM. Predictive and prognostic value of the 21-gene recurrence score in hormone receptor-positive, node-positive breast cancer. *American journal of clinical oncology*. 2014 Aug;37(4):404-10. PMID: 24853663
33. Nitz, U, Gluz, O, Christgen, M, et al. Reducing chemotherapy use in clinically high-risk, genomically low-risk pN0 and pN1 early breast cancer patients: five-year data from the prospective, randomised phase 3 West German Study Group (WSG) PlanB trial. *Breast Cancer Res Treat*. 2017 Oct;165(3):573-83. PMID: 28664507
34. Gluz, O, Nitz, UA, Christgen, M, et al. West German Study Group Phase III PlanB Trial: First Prospective Outcome Data for the 21-Gene Recurrence Score Assay and Concordance of Prognostic Markers by Central and Local Pathology Assessment. *J Clin Oncol*. 2016;34:2341-9. PMID: 26926676
35. Ueno, T, Masuda, N, Yamanaka, T, et al. Evaluating the 21-gene assay Recurrence Score(R) as a predictor of clinical response to 24 weeks of neoadjuvant exemestane in estrogen receptor-positive breast cancer. *International journal of clinical oncology*. 2014 Aug;19(4):607-13. PMID: 24101215
36. Markopoulos, C, Xepapadakis, G, Venizelos, V, et al. Clinical experience of using oncotype DX as an additional treatment decision tool in early breast cancer - A retrospective analysis from 5 Greek institutions. *Eur J Surg Oncol*. 2012 May;38(5):413-9. PMID: 22425282
37. Albain, KS, Barlow, WE, Shak, S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. *Lancet Oncol*. 2010 Jan;11(1):55-65. PMID: 20005174
38. Baehner, FL, Butler, SM, Yoshizawa, CN. The development of the DCIS score: Scaling and normalization in the Marin General Hospital cohort. *J Clin Oncol*. 2012;30(Suppl 27):Abstr 190. PMID: No PMID Entry
39. Rakovitch, E, Nofech-Mozes, S, Hanna, W, et al. A population-based validation study of the DCIS Score predicting recurrence risk in individuals treated by breast-conserving surgery alone. *Breast Cancer Res Treat*. 2015 Jul;152(2):389-98. PMID: 26119102

40. Solin, LJ, Gray, R, Baehner, FL, et al. A multigene expression assay to predict local recurrence risk for ductal carcinoma in situ of the breast. *J Natl Cancer Inst*. 2013;105:701-10. PMID: 23641039
41. Rakovitch, E, Gray, R, Baehner, FL, et al. Refined estimates of local recurrence risks by DCIS score adjusting for clinicopathological features: a combined analysis of ECOG-ACRIN E5194 and Ontario DCIS cohort studies. *Breast Cancer Res Treat*. 2018 Jun;169(2):359-69. PMID: 29388015
42. Badve, SS, Baehner, FL, Gray, RP, et al. Estrogen- and progesterone-receptor status in ECOG 2197: comparison of immunohistochemistry by local and central laboratories and quantitative reverse transcription polymerase chain reaction by central laboratory. *J Clin Oncol*. 2008 May 20;26(15):2473-81. PMID: 18487567
43. Khoury, T, Yan, L, Liu, S, Bshara, W. Oncotype DX RT-qPCR assay for ER and PR correlation with IHC: a study of 3 different clones. *Appl Immunohistochem Mol Morphol*. 2015 Mar;23(3):178-87. PMID: 24992175
44. Wolff, AC, Hammond, ME, Schwartz, JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *J Clin Oncol*. 2007 Jan 1;25(1):118-45. PMID: 17159189
45. Baehner, FL, Achacoso, N, Maddala, T, et al. Human epidermal growth factor receptor 2 assessment in a case-control study: comparison of fluorescence in situ hybridization and quantitative reverse transcription polymerase chain reaction performed by central laboratories. *J Clin Oncol*. 2010 Oct 1;28(28):4300-6. PMID: 20697093
46. Cardoso, F, van't Veer, LJ, Bogaerts, J, et al. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *N Engl J Med*. 2016 Aug 25;375(8):717-29. PMID: 27557300
47. Cusumano, PG, Generali, D, Ciruelos, E, et al. European inter-institutional impact study of MammaPrint. *Breast*. 2014 Aug;23(4):423-8. PMID: 24685596
48. Esserman, LJ, Yau, C, Thompson, CK, et al. Use of Molecular Tools to Identify Patients With Indolent Breast Cancers With Ultralow Risk Over 2 Decades. *JAMA oncology*. 2017 Nov 1;3(11):1503-10. PMID: 28662222
49. van 't Veer, LJ, Yau, C, Yu, NY, et al. Tamoxifen therapy benefit for patients with 70-gene signature high and low risk. *Breast Cancer Res Treat*. 2017 Nov;166(2):593-601. PMID: 28776283
50. Groenendijk, FH, Jager, A, Cardoso, F, van Deurzen, CHM. A nationwide registry-based cohort study of the MammaPrint genomic risk classifier in invasive breast cancer. *Breast*. 2018 Jan 5;38:125-31. PMID: 29310037
51. Sapino, A, Roepman, P, Linn, SC, et al. MammaPrint molecular diagnostics on formalin-fixed, paraffin-embedded tissue. *J Mol Diagn*. 2014 Mar;16(2):190-7. PMID: 24378251
52. Drukker, CA, Bueno-de-Mesquita, JM, Retel, VP, et al. A prospective evaluation of a breast cancer prognosis signature in the observational RASTER study. *Int J Cancer*. 2013 Aug 15;133(4):929-36. PMID: 23371464
53. Saghatchian, M, Mook, S, Pruneri, G, et al. Additional prognostic value of the 70-gene signature (MammaPrint((R))) among breast cancer patients with 4-9 positive lymph nodes. *Breast*. 2013 Oct;22(5):682-90. PMID: 23347730
54. Ahn, SG, Lee, HM, Lee, HW, et al. Prognostic discrimination using a 70-gene signature among patients with estrogen receptor-positive breast cancer and an intermediate 21-gene recurrence score. *International journal of molecular sciences*. 2013;14(12):23685-99. PMID: 24304542

55. Esserman, LJ, Berry, DA, Cheang, MC, et al. Chemotherapy response and recurrence-free survival in neoadjuvant breast cancer depends on biomarker profiles: results from the I-SPY 1 TRIAL (CALGB 150007/150012; ACRIN 6657). *Breast Cancer Res Treat*. 2012 Apr;132(3):1049-62. PMID: 22198468
56. Wittner, BS, Sgroi, DC, Ryan, PD, et al. Analysis of the MammaPrint breast cancer assay in a predominantly postmenopausal cohort. *Clin Cancer Res*. 2008 May 15;14(10):2988-93. PMID: 18483364
57. Bueno-de-Mesquita, JM, Linn, SC, Keijzer, R, et al. Validation of 70-gene prognosis signature in node-negative breast cancer. *Breast Cancer Res Treat*. 2009 Oct;117(3):483-95. PMID: 18819002
58. Ma, XJ, Wang, Z, Ryan, PD, et al. A two-gene expression ratio predicts clinical outcome in breast cancer patients treated with tamoxifen. *Cancer Cell*. 2004 Jun;5(6):607-16. PMID: 15193263
59. van de Vijver, MJ, He, YD, van't Veer, LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med*. 2002 Dec 19;347(25):1999-2009. PMID: 12490681
60. Mook, S, Schmidt, MK, Viale, G, et al. The 70-gene prognosis-signature predicts disease outcome in breast cancer patients with 1-3 positive lymph nodes in an independent validation study. *Breast Cancer Res Treat*. 2009 Jul;116(2):295-302. PMID: 18661261
61. Rutgers, E, Piccart-Gebhart, MJ, Bogaerts, J, et al. The EORTC 10041/BIG 03-04 MINDACT trial is feasible: results of the pilot phase. *Eur J Cancer*. 2011 Dec;47(18):2742-9. PMID: 22051734
62. Knauer, M, Cardoso, F, Wesseling, J, et al. Identification of a low-risk subgroup of HER-2-positive breast cancer by the 70-gene prognosis signature. *Br J Cancer*. 2010 Dec 7;103(12):1788-93. PMID: 21081926
63. Kunz, G. Use of a genomic test (MammaPrint) in daily clinical practice to assist in risk stratification of young breast cancer patients. *Arch Gynecol Obstet*. 2011 Mar;283(3):597-602. PMID: 20383789
64. Knauer, M, Mook, S, Rutgers, EJ, et al. The predictive value of the 70-gene signature for adjuvant chemotherapy in early breast cancer. *Breast Cancer Res Treat*. 2010 Apr;120(3):655-61. PMID: 20204499
65. Ishitobi, M, Goranova, TE, Komoike, Y, et al. Clinical utility of the 70-gene MammaPrint profile in a Japanese population. *Jpn J Clin Oncol*. 2010 Jun;40(6):508-12. PMID: 20110242
66. Mook, S, Knauer, M, Bueno-de-Mesquita, JM, et al. Metastatic potential of T1 breast cancer can be predicted by the 70-gene MammaPrint signature. *Ann Surg Oncol*. 2010 May;17(5):1406-13. PMID: 20094918
67. Bighin, C, Del Mastro, L, Canavese, G, et al. Use in current clinical practice of 70-gene signature in early breast cancer. *Int J Cancer*. 2010 Dec 1;127(11):2736-7. PMID: 20162670
68. Mook, S, Schmidt, MK, Weigelt, B, et al. The 70-gene prognosis signature predicts early metastasis in breast cancer patients between 55 and 70 years of age. *Ann Oncol*. 2010 Apr;21(4):717-22. PMID: 19825882
69. Pohl, H, Kotze, MJ, Grant, KA, et al. Impact of MammaPrint on Clinical Decision-Making in South African Patients with Early-Stage Breast Cancer. *The breast journal*. 2016 Jul;22(4):442-6. PMID: 27079770

70. Exner, R, Bago-Horvath, Z, Bartsch, R, et al. The multigene signature MammaPrint impacts on multidisciplinary team decisions in ER+, HER2- early breast cancer. *Br J Cancer*. 2014;111:837-42. PMID: 25003667
71. Drukker, CA, van den Hout, HC, Sonke, GS, et al. Risk estimations and treatment decisions in early stage breast cancer: agreement among oncologists and the impact of the 70-gene signature. *Eur J Cancer*. 2014 Apr;50(6):1045-54. PMID: 24529927
72. Drukker, CA, Elias, SG, Nijenhuis, MV, et al. Gene expression profiling to predict the risk of locoregional recurrence in breast cancer: a pooled analysis. *Breast Cancer Res Treat*. 2014 Dec;148(3):599-613. PMID: 25414025
73. Tsai, M, Lo, S, Audeh, W, et al. Association of 70-Gene Signature Assay Findings With Physicians' Treatment Guidance for Patients With Early Breast Cancer Classified as Intermediate Risk by the 21-Gene Assay. *JAMA oncology*. 2018 Jan 11;4(1):e173470. PMID: 29075751
74. Peto, R, Davies, C, Godwin, J, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet*. 2012 Feb 4;379(9814):432-44. PMID: 22152853
75. Schroeder, B, Zhang, Y, Stal, O, et al. Risk stratification with Breast Cancer Index for late distant recurrence in patients with clinically low-risk (T1N0) estrogen receptor-positive breast cancer. *NPJ breast cancer*. 2017;3:28. PMID: 28795152
76. Sgroi, DC, Chapman, JA, Badovinac-Crnjevic, T, et al. Assessment of the prognostic and predictive utility of the Breast Cancer Index (BCI): an NCIC CTG MA.14 study. *Breast Cancer Res*. 2016;18(1):1. PMID: 26728744
77. Sgroi, DC, Sestak, I, Cuzick, J, et al. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. *Lancet Oncol*. 2013 Oct;14(11):1067-76. PMID: 24035531
78. Zhang, Y, Schnabel, CA, Schroeder, BE, et al. Breast cancer index identifies early-stage estrogen receptor-positive breast cancer patients at risk for early- and late-distant recurrence. *Clin Cancer Res*. 2013 Aug 1;19(15):4196-205. PMID: 23757354
79. Jerevall, PL, Ma, XJ, Li, H, et al. Prognostic utility of HOXB13:IL17BR and molecular grade index in early-stage breast cancer patients from the Stockholm trial. *Br J Cancer*. 2011 May 24;104(11):1762-9. PMID: 21559019
80. Jankowitz, RC, Cooper, K, Erlander, MG, et al. Prognostic utility of the breast cancer index and comparison to Adjuvant! Online in a clinical case series of early breast cancer. *Breast Cancer Res*. 2011;13(5):R98. PMID: 21999244
81. Ma, XJ, Salunga, R, Dahiya, S, et al. A five-gene molecular grade index and HOXB13:IL17BR are complementary prognostic factors in early stage breast cancer. *Clin Cancer Res*. 2008 May 1;14(9):2601-8. PMID: 18451222
82. Stephen, J, Murray, G, Cameron, DA, et al. Time dependence of biomarkers: non-proportional effects of immunohistochemical panels predicting relapse risk in early breast cancer. *Br J Cancer*. 2014 Dec 9;111(12):2242-7. PMID: 25314051
83. Bartlett, JM, Thomas, J, Ross, DT, et al. Mammostrat as a tool to stratify breast cancer patients at risk of recurrence during endocrine therapy. *Breast Cancer Res*. 2010;12(4):R47. PMID: 20615243
84. Ross, DT, Kim, CY, Tang, G, et al. Chemosensitivity and stratification by a five monoclonal antibody immunohistochemistry test in the NSABP B14 and B20 trials. *Clin Cancer Res*. 2008 Oct 15;14(20):6602-9. PMID: 18927301

85. Ring, BZ, Seitz, RS, Beck, R, et al. Novel prognostic immunohistochemical biomarker panel for estrogen receptor-positive breast cancer. *J Clin Oncol*. 2006 Jul 1;24(19):3039-47. PMID: 16809728
86. Tutt, A, Wang, A, Rowland, C, et al. Risk estimation of distant metastasis in node-negative, estrogen receptor-positive breast cancer patients using an RT-PCR based prognostic expression signature. *BMC Cancer*. 2008;8:339. PMID: 19025599
87. Welsh, AW, Moeder, CB, Kumar, S, et al. Standardization of estrogen receptor measurement in breast cancer suggests false-negative results are a function of threshold intensity rather than percentage of positive cells. *J Clin Oncol*. 2011 Aug 1;29(22):2978-84. PMID: 21709197
88. Allred, DC, Carlson, RW, Berry, DA, et al. NCCN Task Force Report: Estrogen Receptor and Progesterone Receptor Testing in Breast Cancer by Immunohistochemistry. *Journal of the National Comprehensive Cancer Network : JNCCN*. 2009 Sep;7 Suppl 6:S1-S21; quiz S2-3. PMID: 19755043
89. Cuzick, J, Dowsett, M, Pineda, S, et al. Prognostic value of a combined estrogen receptor, progesterone receptor, Ki-67, and human epidermal growth factor receptor 2 immunohistochemical score and comparison with the Genomic Health recurrence score in early breast cancer. *J Clin Oncol*. 2011 Nov 10;29(32):4273-8. PMID: 21990413
90. Barton, S, Zabaglo, L, A'Hern, R, et al. Assessment of the contribution of the IHC4+C score to decision making in clinical practice in early breast cancer. *Br J Cancer*. 2012 May 22;106(11):1760-5. PMID: 22531639
91. Geiss, GK, Bumgarner, RE, Birditt, B, et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. *Nature biotechnology*. 2008 Mar;26(3):317-25. PMID: 18278033
92. Sestak, I, Cuzick, J, Dowsett, M, et al. Prediction of late distant recurrence after 5 years of endocrine treatment: a combined analysis of patients from the Austrian breast and colorectal cancer study group 8 and arimidex, tamoxifen alone or in combination randomized trials using the PAM50 risk of recurrence score. *J Clin Oncol*. 2015 Mar 10;33(8):916-22. PMID: 25332252
93. Gnant, M, Sestak, I, Filipits, M, et al. Identifying clinically relevant prognostic subgroups of postmenopausal women with node-positive hormone receptor-positive early-stage breast cancer treated with endocrine therapy: a combined analysis of ABCSG-8 and ATAC using the PAM50 risk of recurrence score and intrinsic subtype. *Ann Oncol*. 2015 May 1. PMID: 25935792
94. Gnant, M, Filipits, M, Greil, R, et al. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 Risk of Recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. *Ann Oncol*. 2014 Feb;25(2):339-45. PMID: 24347518
95. Ohnstad, HO, Borgen, E, Falk, RS, et al. Prognostic value of PAM50 and risk of recurrence score in patients with early-stage breast cancer with long-term follow-up. *Breast Cancer Res*. 2017 Nov 14;19(1):120. PMID: 29137653
96. Liu, S, Chapman, JA, Burnell, MJ, et al. Prognostic and predictive investigation of PAM50 intrinsic subtypes in the NCIC CTG MA.21 phase III chemotherapy trial. *Breast Cancer Res Treat*. 2015 Jan;149(2):439-48. PMID: 25552364
97. Cheang, MC, Voduc, KD, Tu, D, et al. Responsiveness of intrinsic subtypes to adjuvant anthracycline substitution in the NCIC.CTG MA.5 randomized trial. *Clin Cancer Res*. 2012 Apr 15;18(8):2402-12. PMID: 22351696

98. Nielsen, T, Wallden, B, Schaper, C, et al. Analytical validation of the PAM50-based Prosigna Breast Cancer Prognostic Gene Signature Assay and nCounter Analysis System using formalin-fixed paraffin-embedded breast tumor specimens. *BMC Cancer*. 2014;14:177. PMID: 24625003
99. Filipits, M, Nielsen, TO, Rudas, M, et al. The PAM50 risk-of-recurrence score predicts risk for late distant recurrence after endocrine therapy in postmenopausal women with endocrine-responsive early breast cancer. *Clin Cancer Res*. 2014 Mar 1;20(5):1298-305. PMID: 24520097
100. Dowsett, M, Sestak, I, Lopez-Knowles, E, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. *J Clin Oncol*. 2013 Aug 1;31(22):2783-90. PMID: 23816962
101. Sestak, I, Dowsett, M, Zabaglo, L, et al. Factors predicting late recurrence for estrogen receptor-positive breast cancer. *J Natl Cancer Inst*. 2013 Oct 2;105(19):1504-11. PMID: 24029245
102. Nielsen, TO, Parker, JS, Leung, S, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. *Clin Cancer Res*. 2010 Nov 1;16(21):5222-32. PMID: 20837693
103. Parker, JS, Mullins, M, Cheang, MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol*. 2009 Mar 10;27(8):1160-7. PMID: 19204204
104. Kimbung, S, Markholm, I, Bjohle, J, et al. Assessment of early response biomarkers in relation to long-term survival in patients with HER2-negative breast cancer receiving neoadjuvant chemotherapy plus bevacizumab: Results from the Phase II PROMIX trial. *Int J Cancer*. 2018 Feb 1;142(3):618-28. PMID: 28940389
105. Laenkholm, AV, Jensen, MB, Eriksen, JO, et al. The ability of PAM50 risk of recurrence score to predict 10-year distant recurrence in hormone receptor-positive postmenopausal women with special histological subtypes. *Acta oncologica (Stockholm, Sweden)*. 2018 Jan;57(1):44-50. PMID: 29202609
106. Laurberg, T, Tramm, T, Nielsen, T, et al. Intrinsic subtypes and benefit from postmastectomy radiotherapy in node-positive premenopausal breast cancer patients who received adjuvant chemotherapy - results from two independent randomized trials. *Acta oncologica (Stockholm, Sweden)*. 2018 Jan;57(1):38-43. PMID: 29172851
107. Tobin, NP, Lundberg, A, Lindstrom, LS, et al. PAM50 Provides Prognostic Information When Applied to the Lymph Node Metastases of Advanced Breast Cancer Patients. *Clin Cancer Res*. 2017 Dec 1;23(23):7225-31. PMID: 28972041
108. Sanchez-Munoz, A, Vicioso, L, Santonja, A, et al. Male breast cancer: correlation between immunohistochemical subtyping and PAM50 intrinsic subtypes, and the subsequent clinical outcomes. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc*. 2017 Oct 6. PMID: 28984296
109. Kim, HK, Park, KH, Kim, Y, et al. Discordance of the PAM50 Intrinsic Subtypes Compared with IHC-Based Surrogate in Breast Cancer Patients: Potential Implication of Genomic Alterations of Discordance. *Cancer Res Treat*. 2018;5(342):342. PMID:
110. Hequet, D, Callens, C, Gentien, D, et al. Prospective, multicenter French study evaluating the clinical impact of the Breast Cancer Intrinsic Subtype-Prosigna(R) Test in the management of early-stage breast cancers. *PLoS One*. 2017;12(10):e0185753. PMID: 29045452
111. Martin, M, Gonzalez-Rivera, M, Morales, S, et al. Prospective study of the impact of the Prosigna assay on adjuvant clinical decision-making in unselected patients with estrogen receptor positive, human epidermal growth factor receptor negative, node

negative early-stage breast cancer. *Current medical research and opinion*. 2015 Jun;31(6):1129-37. PMID: 25851308
112. Wesseling, J, Tinterri, C, Sapino, A, et al. An international study comparing conventional versus mRNA level testing (TargetPrint) for ER, PR, and HER2 status of breast cancer. *Virchows Archiv : an international journal of pathology*. 2016 Sep;469(3):297-304. PMID: 27377889
113. Grant, KA, Pienaar, FM, Brundyn, K, et al. Incorporating microarray assessment of HER2 status in clinical practice supports individualised therapy in early-stage breast cancer. *Breast*. 2015 Apr;24(2):137-42. PMID: 25586984
114. Whitworth, P, Stork-Sloots, L, de Snoo, FA, et al. Chemosensitivity predicted by BluePrint 80-gene functional subtype and MammaPrint in the Prospective Neoadjuvant Breast Registry Symphony Trial (NBRST). *Ann Surg Oncol*. 2014 Oct;21(10):3261-7. PMID: 25099655
115. Viale, G, Slaets, L, Bogaerts, J, et al. High concordance of protein (by IHC), gene (by FISH; HER2 only), and microarray readout (by TargetPrint) of ER, PgR, and HER2: results from the EORTC 10041/BIG 03-04 MINDACT trial. *Ann Oncol*. 2014 Apr;25(4):816-23. PMID: 24667714
116. Nguyen, B, Cusumano, PG, Deck, K, et al. Comparison of molecular subtyping with BluePrint, MammaPrint, and TargetPrint to local clinical subtyping in breast cancer patients. *Ann Surg Oncol*. 2012 Oct;19(10):3257-63. PMID: 22965266
117. Krijgsman, O, Roepman, P, Zwart, W, et al. A diagnostic gene profile for molecular subtyping of breast cancer associated with treatment response. *Breast Cancer Res Treat*. 2012 May;133(1):37-47. PMID: 21814749
118. Van Laar, RK. Design and multiseries validation of a web-based gene expression assay for predicting breast cancer recurrence and patient survival. *J Mol Diagn*. 2011;13:297-304. PMID: 21458382
119. D'Alfonso, TM, van Laar, RK, Vahdat, LT, et al. BreastPRS is a gene expression assay that stratifies intermediate-risk Oncotype DX patients into high- or low-risk for disease recurrence. *Breast Cancer Res Treat*. 2013 Jun;139(3):705-15. PMID: 23774991
120. Filipits, M, Rudas, M, Jakesz, R, et al. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. *Clin Cancer Res*. 2011 Sep 15;17(18):6012-20. PMID: 21807638
121. Buus, R, Sestak, I, Kronenwett, R, et al. Comparison of EndoPredict and EPclin With Oncotype DX Recurrence Score for Prediction of Risk of Distant Recurrence After Endocrine Therapy. *J Natl Cancer Inst*. 2016 Nov;108(11). PMID: 27400969
122. Bertucci, F, Finetti, P, Viens, P, Birnbaum, D. EndoPredict predicts for the response to neoadjuvant chemotherapy in ER-positive, HER2-negative breast cancer. *Cancer letters*. 2014 Dec 1;355(1):70-5. PMID: 25218596
123. Martin, M, Brase, JC, Calvo, L, et al. Clinical validation of the EndoPredict test in node-positive, chemotherapy-treated ER+/HER2- breast cancer patients: results from the GEICAM 9906 trial. *Breast Cancer Res*. 2014;16(2):R38. PMID: 24725534
124. Dubsky, P, Brase, JC, Jakesz, R, et al. The EndoPredict score provides prognostic information on late distant metastases in ER+/HER2- breast cancer patients. *Br J Cancer*. 2013 Dec 10;109(12):2959-64. PMID: 24157828
125. Fitzal, F, Filipits, M, Rudas, M, et al. The genomic expression test EndoPredict is a prognostic tool for identifying risk of local recurrence in postmenopausal endocrine receptor-positive, her2neu-negative breast cancer patients randomised within the prospective ABCSG 8 trial. *Br J Cancer*. 2015 Apr 14;112(8):1405-10. PMID: 25867274

126. Chow, LWC, Morita, S, Chow, CYC, Ng, WK, Toi, M. Neoadjuvant palbociclib on ER+ breast cancer (N007): clinical response and EndoPredict's value. *Endocrine-related cancer*. 2018 Feb;25(2):123-30. PMID: 29158285
127. Mokbel, K, Wazir, U, El Hage Chehade, H, Manson, A, Choy, C, Moye, V. A Comparison of the Performance of EndoPredict Clinical and NHS PREDICT in 120 Patients Treated for ER-positive Breast Cancer. *Anticancer research*. 2017 Dec;37(12):6863-9. PMID: 29187466
128. Blok, EJ, Bastiaannet, E, van den Hout, WB, et al. Systematic review of the clinical and economic value of gene expression profiles for invasive early breast cancer available in Europe. *Cancer treatment reviews*. 2018 Jan;62:74-90. PMID: 29175678
129. Chang, MC, Souter, LH, Kamel-Reid, S, et al. Clinical utility of multigene profiling assays in early-stage breast cancer. *Current oncology (Toronto, Ont)*. 2017 Oct;24(5):e403-e22. PMID: 29089811
130. Sestak, I, Buus, R, Cuzick, J, et al. Comparison of the Performance of 6 Prognostic Signatures for Estrogen Receptor-Positive Breast Cancer: A Secondary Analysis of a Randomized Clinical Trial. *JAMA oncology*. 2018 Apr 1;4(4):545-53. PMID: 29450494
131. Bosl, A, Spitzmuller, A, Jasarevic, Z, Rauch, S, Jager, S, Offner, F. MammaPrint versus EndoPredict: Poor correlation in disease recurrence risk classification of hormone receptor positive breast cancer. *PLoS One*. 2017;12(8):e0183458. PMID: 28850621
132. Lundberg, A, Lindstrom, LS, Harrell, JC, et al. Gene Expression Signatures and Immunohistochemical Subtypes Add Prognostic Value to Each Other in Breast Cancer Cohorts. *Clin Cancer Res*. 2017 Dec 15;23(24):7512-20. PMID: 28972043
133. Sestak, I, Zhang, Y, Schroeder, BE, et al. Cross-Stratification and Differential Risk by Breast Cancer Index and Recurrence Score in Women with Hormone Receptor-Positive Lymph Node-Negative Early-Stage Breast Cancer. *Clin Cancer Res*. 2016 Oct 15;22(20):5043-8. PMID: 27252417
134. Hornberger, J, Alvarado, MD, Rebecca, C, Gutierrez, HR, Yu, TM, Gradishar, WJ. Clinical validity/utility, change in practice patterns, and economic implications of risk stratifiers to predict outcomes for early-stage breast cancer: a systematic review. *J Natl Cancer Inst*. 2012;104:1068-79. PMID: 22767204
135. Varga, Z, Sinn, P, Fritzsche, F, et al. Comparison of EndoPredict and Oncotype DX test results in hormone receptor positive invasive breast cancer. *PLoS One*. 2013;8:e58483. PMID: 23505515
136. Fan, C, Oh, DS, Wessels, L, et al. Concordance among gene-expression-based predictors for breast cancer. *N Engl J Med*. 2006 Aug 10;355(6):560-9. PMID: 16899776
137. Espinosa, E, Vara, JA, Redondo, A, et al. Breast cancer prognosis determined by gene expression profiling: a quantitative reverse transcriptase polymerase chain reaction study. *J Clin Oncol*. 2005 Oct 10;23(29):7278-85. PMID: 16129846
138. Kelly, CM, Bernard, PS, Krishnamurthy, S, et al. Agreement in risk prediction between the 21-gene recurrence score assay (Oncotype DX(R)) and the PAM50 breast cancer intrinsic Classifier in early-stage estrogen receptor-positive breast cancer. *Oncologist*. 2012;17(4):492-8. PMID: 22418568
139. Harris, LN, Ismaila, N, McShane, LM, et al. Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol*. 2016;34:1134-50. PMID: 26858339
140. Hammond, ME, Hayes, DF, Wolff, AC, Mangu, PB, Temin, S. American society of clinical oncology/college of american pathologists guideline recommendations for

immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J Oncol Pract*. 2010 Jul;6(4):195-7. PMID: 21037871
141. Wolff, AC, Hammond, ME, Hicks, DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/college of American Pathologists clinical practice guideline update. *Archives of pathology & laboratory medicine*. 2014 Feb;138(2):241-56. PMID: 24099077
142. U.S. Preventive Services Task Force. Final Recommendation Statement. BRCA-related Cancer: Risk Assessment, Genetic Counseling, and Genetic Testing. [cited 01/03/2019]; Available from: http://www.uspreventiveservicestaskforce.org/Page/Document/RecommendationStatementFinal/brca-related-cancer-risk-assessment-genetic-counseling-and-genetic-testing
143. BlueCross BlueShield Association Medical Policy Reference Manual "Assays of Genetic Expression in Tumor Tissue as a Technique to Determine Prognosis In Patients With Breast Cancer." Policy No. 2.04.36

CODES

Codes	Number	Description
CPT	~~0008M~~	~~Oncology (breast), mRNA analysis of 58 genes using hybrid capture, on formalin-fixed paraffin-embedded (FFPE) tissue, prognostic algorithm reported as a risk score~~ (Deleted 1/1/2018)
	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
	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
HCPCS	S3854	Gene expression profiling panel for use in the management of breast cancer treatment

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