



Name of Policy:

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

Policy #: 180

Category: Medicine/Laboratory/Pathology

Latest Review Date: July 2014

Policy Grade: B

Background/Definitions:

As a general rule, benefits are payable under Blue Cross and Blue Shield of Alabama health plans only in cases of medical necessity and only if services or supplies are not investigational, provided the customer group contracts have such coverage.

The following Association Technology Evaluation Criteria must be met for a service/supply to be considered for coverage:

- 1. The technology must have final approval from the appropriate government regulatory bodies;*
- 2. The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes;*
- 3. The technology must improve the net health outcome;*
- 4. The technology must be as beneficial as any established alternatives;*
- 5. The improvement must be attainable outside the investigational setting.*

Medical Necessity means that health care services (e.g., procedures, treatments, supplies, devices, equipment, facilities or drugs) that a physician, exercising prudent clinical judgment, would provide to a patient for the purpose of preventing, evaluating, diagnosing or treating an illness, injury or disease or its symptoms, and that are:

- 1. In accordance with generally accepted standards of medical practice; and*
- 2. Clinically appropriate in terms of type, frequency, extent, site and duration and considered effective for the patient's illness, injury or disease; and*
- 3. Not primarily for the convenience of the patient, physician or other health care provider; and*
- 4. Not more costly than an alternative service or sequence of services at least as likely to produce equivalent therapeutic or diagnostic results as to the diagnosis or treatment of that patient's illness, injury or disease.*

Description of Procedure or Service:

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

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

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

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

Test	Manufacturer	Description
Oncotype DX®	Genomic Health	21-gene RT-PCR
MammaPrint®	Agendia	70-gene DNA microarray
<u>Blueprint® TargetPrint®</u>	<u>Agendia</u>	<u>Both tests are intended for use with MammaPrint®: Blueprint®: 80-gene subtype classifier TargetPrint®: quantitative ER, PR, and HER2 microarray</u>
Breast Cancer Index SM	bioTheranostics	Combines MGI and the HOXB13:IL17BR Index
Mammostrat® Breast Cancer Test	Clariant Diagnostic Services	IHC assay of 5 biomarkers independent of tumor proliferation and grade
BreastOncPx™(Breast Cancer Prognosis Gene Expression Assay)	LabCorp	14-gene RT-PCR
NexCourse® Breast IHC4	Geneoptix	IHC assay of ER, PR, HER2, and Ki-67
<u>Prosigna™</u>	<u>NanoString Technologies</u>	<u>DNA microarray based on the PAM50 breast cancer intrinsic subtype classifier</u>

BreastPRS™	Signal Genetics	200-gene assay
EndoPredict™	Sividon Diagnostics	12-gene RT-PCR

ER: estrogen receptor; HER2: human epidermal growth factor receptor 2; IHC: immunohistochemistry; Ki-67: a marker of tumor proliferation; MGI: Molecular Grade Index; PAM50: prediction analysis of microarray 50 gene set; PR: progesterone receptor; RT-PCR: real-time reverse transcriptase-polymerase chain reaction.

Blueprint® and TargetPrint®

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

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

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

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

Policy:

Effective for dates of service on or after July 1, 2014:

The **use of the 21-gene RT-PCR assay** (i.e., Oncotype DX®) to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy **meets** Blue Cross and Blue Shield of Alabama’s medical criteria for coverage in women with breast cancer meeting all of the following characteristics:

- Unilateral, ~~non-fixed~~ tumor;
- Hormone receptor positive (that is ER-positive or PR-positive);
- HER2-negative;
- Tumor size 0.6-1 cm with moderate/poor differentiation or unfavorable features OR tumor size > 1 cm;
- Node negative (lymph nodes with micrometastases (< 2 mm in size) are considered node negative for this policy statement);
- Who will be treated with adjuvant endocrine therapy, e.g., tamoxifen or aromatase inhibitors;
- When the test result will aid the patient in making the decision regarding chemotherapy (i.e., when chemotherapy is a therapeutic option); AND

- When ordered within 6 months following diagnosis, since the value of the test for making decisions regarding delayed chemotherapy is unknown.

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

For patients who otherwise meet the above characteristics but who have multiple ipsilateral primaries, a specimen from the tumor with the most aggressive histological characteristics should be submitted for testing, as this is how prognosis is clinically determined. It is not necessary to conduct Oncotype DX testing on each tumor.

All other indications for the 21-gene RT-PCR assay (i.e., Oncotype DX®) including determination of recurrence risk in breast cancer patients with positive lymph nodes or patients with bilateral disease **do not meet** Blue Cross and Blue Shield of Alabama's medical criteria for coverage and is considered **investigational**.

Use of a subset of genes from the 21-gene RT-PCR assay for predicting recurrence risk in patients with noninvasive ductal carcinoma in situ (i.e., Oncotype DX DCIS) to inform treatment planning following excisional surgery **does not meet** Blue Cross and Blue Shield of Alabama's medical criteria for coverage and is considered **investigational**.

The use of other gene expression assays (including, but not limited to, MammaPrint, Mammostrat, the Breast Cancer Index, the BreastOncPx, NexCourse Breast IHC4, Prosigna, ~~or PAM50 Breast Cancer Intrinsic Classifier BreastPRS, and EndoPredict~~) **for any indication does not meet** Blue Cross and Blue Shield of Alabama's medical criteria for coverage and is considered **investigational**.

The use of gene expression assays in men with breast cancer does not meet Blue Cross and Blue Shield of Alabama's medical criteria and is considered **investigational**.

The use of gene expression assays to molecularly subclassify breast cancer (e.g., Blueprint®) does not meet Blue Cross and Blue Shield of Alabama's medical criteria and is considered **investigational**.

The use of gene expression assays for quantitative assessment of ER, PR, and HER2 overexpression (e.g., TargetPrint®) does not meet Blue Cross and Blue Shield of Alabama's medical criteria and is considered **investigational**.

Effective for dates of service prior to July 1, 2014:

The **use of the 21-gene RT-PCR assay** (i.e., Oncotype DX®) to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy **meets** Blue Cross and Blue Shield of Alabama's medical criteria for coverage in women with breast cancer meeting all of the following characteristics:

- Unilateral, non-fixed tumor;
- Hormone receptor positive (that is ER-positive or PR-positive);
- HER2-negative;
- Tumor size 0.6-1 cm with moderate/poor differentiation or unfavorable features OR tumor size > 1 cm;
- Node negative (lymph nodes with micrometastases (< 2 mm in size) are considered node negative for this policy statement);
- Who will be treated with adjuvant endocrine therapy, e.g., tamoxifen or aromatase inhibitors;
- When the test result will aid the patient in making the decision regarding chemotherapy (i.e., when chemotherapy is a therapeutic option); AND
- When ordered within 6 months following diagnosis, since the value of the test for making decisions regarding delayed chemotherapy is unknown.

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

For patients who otherwise meet the above characteristics but who have multiple ipsilateral primaries, a specimen from the tumor with the most aggressive histological characteristics should be submitted for testing, as this is how prognosis is clinically determined. It is not necessary to conduct Oncotype DX testing on each tumor.

All other indications for the 21-gene RT-PCR assay (i.e., Oncotype DX®) including determination of recurrence risk in breast cancer patients with positive lymph nodes or patients with bilateral disease **do not meet** Blue Cross and Blue Shield of Alabama's medical criteria for coverage and is considered **investigational**.

Use of a subset of genes from the 21-gene RT-PCR assay for predicting recurrence risk in patients with noninvasive ductal carcinoma in situ (i.e., Oncotype DX DCIS) to inform treatment planning following excisional surgery **does not meet** Blue Cross and Blue Shield of Alabama's medical criteria for coverage and is considered **investigational**.

The use of other gene expression assays (including, but not limited to, MammaPrint, Mammostrat, the Breast Cancer Index, the BreastOncPx, NexCourse Breast IHC4, or PAM50 Breast Cancer Intrinsic Classifier) **for any indication does not meet** Blue Cross and Blue Shield of Alabama's medical criteria for coverage and is considered **investigational**.

Blue Cross and Blue Shield of Alabama does not approve or deny procedures, services, testing, or equipment for our members. Our decisions concern coverage only. The decision of whether or not to have a certain test, treatment or procedure is one made between the physician and his/her patient. Blue Cross and Blue Shield of Alabama administer benefits based on the member's contract and corporate medical policies. Physicians should always exercise their best medical judgment in providing the care they feel is most appropriate for their patients. Needed care should not be delayed or refused because of a coverage determination.

Key Points:

In 2014, a TEC Assessment addressed gene expression profiling in women with lymph node-negative breast cancer to select adjuvant chemotherapy, specifically use of Oncotype DX®, MammaPrint®, the Breast Cancer IndexSM, and ProsignaTM/PAM50 gene expression assay. The Assessment concluded that use of Oncotype DX® to determine recurrence risk for deciding whether to undergo adjuvant chemotherapy in women with unilateral, hormone receptor-positive, lymph node-negative breast cancer who will receive hormonal therapy meets the Blue Cross and Blue Shield Association Technology Evaluation Center (TEC) criteria; and that use of MammaPrint®, the Breast Cancer IndexSM, and ProsignaTM 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.

This policy evidence review is based on the above TEC Assessments and on published evidence related to the assays listed in the Description.

Oncotype Dx®

Description

The initial indication for the 21-gene expression profile (Oncotype DX®) was for patients newly diagnosed with Stage 1 or 2, lymph node-negative, estrogen receptor (ER)-positive invasive breast cancer who would be treated with tamoxifen. Primary validation studies enrolled node-negative patients; this indication is reviewed first. More recently, Genomic Health has expanded indications for Oncotype DX® to include all Stage 2 disease (tumor ≤2 cm with spread to axillary lymph nodes or 2-5 cm without lymph node involvement) and ductal carcinoma in situ (DCIS); these indications are reviewed separately.

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

Lymph Node-negative Patients

Studies delineating the association between the 21-gene RS and recurrence risk are shown in Table 3. Results indicated strong, independent associations between the RS and distant disease recurrence or death from breast cancer. In secondary reclassification analyses of the Paik et al data, Bryant et al (published in Tang et al) classified individual patient risk levels by conventional risk classifiers, and then reclassified patients by Oncotype DX®. 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 who were actually at lower risk of recurrence (average 7%-9% risk at 10 years; upper 95% confidence interval [CI] limits, 11% to 15%). The analysis did not indicate significant erroneous reclassification given known outcomes. Thus, a woman who prefers to avoid the toxicity and inconvenience of chemotherapy and whose Oncotype DX® RS value shows that she is at very low risk of recurrence might reasonably decline chemotherapy. The lower the RS value, the greater the confidence the woman can have that chemotherapy will not provide net benefit; outcomes are improved by avoiding chemotherapy toxicity.

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

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

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

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

Additionally, a recent meta-analysis indicated that anthracyclines do not improve disease-free survival (DFS) or overall survival (OS) in women with early, HER2-negative breast cancer, and therefore may not be prescribed in this population.

- Lymph nodes with micrometastases are not considered positive for purposes of treatment recommendations. Current practice largely involves a detailed histologic examination of sentinel lymph nodes, allowing for the detection of micrometastases (<2 mm in size).
- Those whose tumors are ER-positive or progesterone receptor (PR)-positive: Only ER-positive women were enrolled in Oncotype DX® validation studies, whereas current clinical guidelines include either ER or PR positivity in the treatment pathway for hormone receptor-positive women with early breast cancer. Some studies have shown that ER-negative/PR-positive patients also tend to benefit from endocrine therapy. Studies documenting the low incidence (1%-4%) and instability (lack of reproducibility) of the ER-negative/PR-positive subtype and the reduction in reports of this subtype with improved assay techniques suggest that this subtype may represent a false negative result.

Papers related to the use of Oncotype DX® that have been published since the 2014 Assessment will be briefly mentioned.

Tzeng et al (2010) examined how women receive and incorporate results of Oncotype DX® using mailed survey and chart review. Approximately two-thirds of women believed they understood most or all of what they were told about their recurrence risk based on their test results; most that experienced test-related distress had intermediate or high estimated recurrence risks by RS result. The objective, recalled, and perceived recurrence risks by women in the study were surprisingly similar, and 95% agreed that the test gave them a better understanding of their treatment options and chances of success. However, approximately one-third of women believed they understood only a moderate amount or less during these discussions. The study was limited in generalizability in that participants were mostly white, well-educated women who had health insurance and came from urban areas.

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

- In a retrospective reclassification analysis, Joh et al (2011) found that addition of Oncotype DX® RS resulted in a 25% change in (after-the-fact) treatment recommendations, resulting in fewer patients projected to receive chemotherapy.
- Hassett et al (2012) evaluated registry data from the National Comprehensive Cancer Network (NCCN) Breast Cancer Outcomes Database Project focusing on women diagnosed with hormone-receptor (HR)-positive Stage 2 to 3 unilateral breast cancer during 2006 to 2008. Compared with women who had Oncotype-determined intermediate-risk cancer, women who had Oncotype-determined high-risk cancers were more likely to receive chemotherapy (odds ratio [OR], 12.0; 95% CI, 6.7 to 21.3), and women with low-risk cancers were less likely to receive chemotherapy (OR=0.1; 95% CI, 0.1 to 0.2).
- Carlson et al (2013) conducted a systematic review of studies of Oncotype DX® used to inform actual adjuvant chemotherapy decisions in ER-positive, lymph node-negative patients with early stage breast cancer. In eight identified studies (total N=1437).

Oncotype DX® RS changed the chemotherapy recommendation based on clinical-pathologic factors in 33% of patients. Compared with Oncotype DX® high risk patients, low risk patients were statistically more likely to follow Oncotype DX®-directed treatment (relative risk [RR], 1.07; 95% CI, 1.01 to 1.14).

Some view these studies as evidence of clinical utility because more patients avoid the toxicity of chemotherapy; however, actual patient outcomes were not reported in these studies. Additionally, none of the studies formalized and described how information was delivered to patients, nor did they evaluate how patient preferences were incorporated into final treatment decisions. Lo et al (2010) conducted a prospective multicenter study that examined both physician and patient treatment selection, as well as impact of RS results on patients' anxiety, quality of life, and satisfaction with choice of treatment. However, the study did not ensure that results were presented in a consistent format for all patients.

Table 3: Summary of Oncotype Dx® RS and recurrence risk studies

Study Study Type	Total N	Study Objective	Results			
			RS Risk	% of Patients		K-M Distant Recurrence at 10 Years, % (95% CI)
Paik 2004 TAM arm of NSABP B-14 RCT	668	Predict recurrence	Low (<18) Int (18-30) High (>31) All	51 22 27 100		6.8 (4.0 to 9.6) 14.3 (8.3 to 20.3) 30.5 (23.6 to 37.4) 15 (12.5 to 17.9)
Paik 2004 Additional analysis of Paik 2004 data	668	Reclassification study; determine incremental risk vs conventional classifier	Risk Classification by NCCN^a Low (8%) High (92%)	Risk Reclassification by Oncotype DX® Low Int High Low Int High	N 38 12 3 301 137 178 	% DRF at 10 Years (95% CI)^b 100 (NR) 80 (59 to 100) 56 (13 to 100) 93 (89 to 96) 86 (80 to 92) 70 (62 to 77)
Bryant 2005; Tang 2011 Additional analysis of Paik et al 2004 data	668	Reclassification study; determine incremental risk vs conventional classifier	Risk Classification by Adjuvant! Online^a Low (53%) Int-High (47%)	Risk Reclassification by Oncotype DX® Low Int-High Low Int-High	N 214 140 120 194	% Recurrence at 10 Years (95% CI)^b (2.5 to 9) 12.9 (7 to 19) 8.9 (4 to 14) 30.7 (24 to 38)
Habel 2006 Case control	255ER+ TAM+; 361 ER+ TAM-	Predict mortality	RS Risk Low (<18) Int (18-30) High (>31)	10-Year Absolute Risk of Death, % (95% CI) ER+, TAM- Treated 2.8 (1.7 to 3.9) 10.7 (6.3 to 14.9) 15.5 (7.6 to 22.8)		ER+, No TAM 6.2 (4.5 to 7.9) 17.8 (11.8 to 23.3) 19.9 (14.2 to 25.2)

DRF: distant recurrence-free; ER: estrogen receptor; Int: intermediate; K-M: Kaplan Meier; NCCN: National Comprehensive Cancer Network (2004); NR: not reported; NSABP: National Surgical Adjuvant Breast and Bowel Project; RCT: randomized controlled trial; RS: Oncotype DX® recurrence score; TAM: tamoxifen.

^a Percentages are percent of total N.

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

Lymph Node-Positive Patients

Albain et al (2010) evaluated samples from the Southwest Oncology Group Trial 8814, in which randomized node-positive, ER-positive patients treated with tamoxifen for five years were compared with those treated with cyclophosphamide, doxorubicin, fluorouracil (CAF) chemotherapy followed by tamoxifen (CAF-T) for five years. Samples were available for determination of RS for 41% (n=148) and 39% (n=219) of the trial arms, respectively.

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

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

Dowsett et al (2010) included a separate evaluation of node-positive patients in their examination of the ATAC trial samples. Of 306 node-positive patients, 243 had one to three involved nodes, and 63 had four or more; these were not evaluated separately. Rates of distant recurrence at nine years were 17% (95% CI, 12% to 24%), 28% (95% CI, 20% to 39%), and 49% (95% CI, 35% to 64%), in low, intermediate, and high RS risk groups, respectively. It is unclear that the risk of distant recurrence in low-risk RS patients would be sufficiently low to forgo the choice of chemotherapy. The authors note that their study “did not directly evaluate the value of RS in predicting the benefit of chemotherapy.”

Goldstein et al (2008) evaluated samples from the Eastern Cooperative Oncology Group E2197 trial, which included patients with 0 to 3 positive lymph nodes and operable tumor greater than 1cm. Patients were randomly assigned to doxorubicin plus cyclophosphamide or docetaxel plus five years of endocrine therapy; outcomes were not significantly different for the study arms. A case-control study of samples from this trial found that low-risk RS patients with 0 to 1 positive nodes had a recurrence risk of 3.3% (95% CI, 2.2 to 5), and low-risk patients with 2 to 3 positive nodes had a recurrence risk of 7.9% (95% CI, 4.3 to 14.1). RS also was a significant predictor of risk regardless of nodal status.

Chang et al (2008) reported that in women with locally advanced breast cancer treated with neoadjuvant docetaxel (N=97), a complete response was more likely in those with a high RS ($p=0.008$). Gianni et al (2005) studied 93 patients with locally advanced breast cancer who

received neoadjuvant taxane chemotherapy, then postsurgery CMF treatment and tamoxifen (if ER-positive). Pathologic complete response was more likely in patients with high RS than with low RS ($p < 0.01$).

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

Patients with DCIS

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

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

According to the Oncotype website, analyses from the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-14 study and a case-control study by Habel et al (2006) were used to select genes that predict the risk of recurrence independent of tamoxifen treatment and ER status. In a retrospective analysis of data and samples from patients in the prospective Eastern Cooperative Oncology Group E5194 study, the Oncotype DX® Score for DCIS was compared with 10-year recurrence risk in a subset of DCIS patients treated only with surgery and some with tamoxifen ($n=327$). Oncotype DX® DCIS Score was significantly associated with recurrence outcomes (HR=2.31; 95% CI, 1.15 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. These studies addressed the development of the Oncotype DX® DCIS Score and clinical validity (association of the test result with recurrence outcomes). Whether women are better categorized as to their recurrence risk by Oncotype DX® DCIS Score compared with standard clinical indicators of risk has not been addressed.

MammaPrint®

MammaPrint, also called the 70-gene signature, is a prognostic test for women with ER-positive or ER-negative, lymph node-negative invasive breast cancer. The 2014 TEC Assessment reviewed available studies and found insufficient evidence to determine whether MammaPrint®

is better than conventional risk assessment tools in predicting recurrence. Limited technical performance evaluation of the commercial version of the assay, using fresh frozen tumor samples, suggested good reproducibility. In 2014, Sapino et al published a validation study of MammaPrint® using FFPE tissue. In a validation set of 221 tumor samples, concordance of FFPE and frozen tissue low- and high-risk classification was 91.5% (95% CI, 86.9 to 94.5). Concordance of repeat analyses of the same tumor was 96%, and interlaboratory reproducibility (i.e., between labs in the Netherlands and in California) was 96%.

Studies of Primarily Node-Negative Disease

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

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

Original validation studies included patients with both node-negative and node-positive disease. Mook et al (2010) retrospectively evaluated 148 consecutive, node-negative, postmenopausal patients, with primarily ER-positive tumors; only 18% received two years of adjuvant tamoxifen and none received chemotherapy. For the 61% with good prognosis, five-year distant metastasis-free survival (DMFS) probability was 93% (95% CI, 87 to 99), whereas for those with poor prognosis, DMFS was 72% (95% CI, 60 to 84). The authors reported on concordance with Adjuvant! Online, but did not conduct a net reclassification analysis to determine additional impact of the MammaPrint® signature on outcomes.

The Microarray Prognostics in Breast Cancer (RASTER) study was designed to assess feasibility of implementation and impact on treatment decisions of the MammaPrint® 70-gene signature, as well as assess recurrence outcomes. Five-year follow-up results of 427 node-negative, early stage breast cancer patients who participated in the RASTER Study and had a 70-gene signature (MammaPrint®) were published in 2013. Use of MammaPrint® to help direct postsurgery treatment decisions were compared with Adjuvant! Online. Patients were aged 18 to 61 years and had a histologically-confirmed, unilateral, unifocal, primary operable, invasive adenocarcinoma of the breast. Median follow-up was 61.6 months. Eighty percent of patients were ER-positive. Discordant risk classifications occurred in 161 (38%) of 427 cases: 124 (29%)

of 427 cases were discordant MammaPrint® low risk and Adjuvant! Online high risk, and 37 (9%) of 427 cases were discordant MammaPrint® high risk and Adjuvant! Online low risk. Use of MammaPrint® reduced the proportion of Adjuvant! Online high-risk patients by 20% (87/427). Five-year distant recurrence-free interval (DRFI) probabilities were excellent for patients who were clinically high risk but had a low-risk score with MammaPrint®, even in the absence of adjuvant systemic therapy. Results of patients receiving adjuvant therapy are presented in Table 4. Results suggested that MammaPrint® is a better prognostic classifier than standard clinical and pathologic classifiers. However, the study had several limitations. Patient numbers are low, and event numbers very low, making firm conclusions difficult. Actual treatment decisions were based on restrictive Dutch guidelines from 2004 and patients' and doctors' preferences. Additionally, Adjuvant! Online risk estimates were calibrated for ten-year outcomes, whereas RASTER outcomes were at five years. Because most clinical relapses in lymph node-negative, ER-positive breast cancers do not occur until five or even ten years after diagnosis, with or without the use of adjuvant therapy, study data should be considered not yet mature.

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

70-gene signature category	Adjuvant! Online Category	AST	5-year DRFI, %, (95% CI)
<u>Low</u>	<u>Low</u>	<u>7/95 (7%)</u>	<u>95.3 (90.9 to 100)</u>
<u>High</u>	<u>Low</u>	<u>32/37 (86%)</u>	<u>100 (100 to 100)</u>
<u>Low</u>	<u>High</u>	<u>54/124 (44%)</u>	<u>98.4 (96.1 to 100)</u>
<u>High</u>	<u>High</u>	<u>166/171 (97%)</u>	<u>89.8 (85.1 to 94.8)</u>

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

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

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

	Low Risk			High Risk		
	% n	Estimate	95% CI	% n	Estimate	95% CI
<u>Adjuvant! Online</u>	<u>56</u>	<u>95.0</u>	<u>90.3 to 99.9</u>	<u>44</u>	<u>100.0</u>	<u>100.0 to 100.0</u>
<u>Nottingham Prognostic Index</u>	<u>97</u>	<u>97.1</u>	<u>94.3 to 100.0</u>	<u>3</u>	<u>100.0</u>	<u>100.0 to 100.0</u>
<u>St. Gallen 2003</u>	<u>37</u>	<u>98.2</u>	<u>94.8 to 100.0</u>	<u>63</u>	<u>96.6</u>	<u>92.8 to 100.0</u>
<u>Dutch National guidelines 2004</u>	<u>96</u>	<u>97.1</u>	<u>94.3 to 100.0</u>	<u>4</u>	<u>100.0</u>	<u>100.0 to 100.0</u>
<u>Dutch National guidelines 2012</u>	<u>53</u>	<u>98.8</u>	<u>96.3 to 100.0</u>	<u>47</u>	<u>95.4</u>	<u>90.4 to 100.0</u>
<u>PREDICT Plus</u>	<u>89</u>	<u>97.6</u>	<u>94.9 to 100.0</u>	<u>11</u>	<u>94.1</u>	<u>83.6 to 100.0</u>

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

Studies of mixed or node-positive disease

In a study of node-positive disease, Mook et al (2009) evaluated 241 patients with one to three positive nodes and primarily ER-positive, HER2-negative tumors treated variably. The 70-gene signature was a significant predictor of outcome. Reclassification analysis using Adjuvant! Online versus MammaPrint® showed significant additional discrimination of outcomes by the gene signature, but all were confounded by heterogeneous patient treatment. This study also updated the results of 106 patients with one to three positive nodes from the validation study, reporting ten-year breast cancer-specific survival of 98% (95% CI, 94 to 100) for good prognosis signatures and 64% (95% CI, 52 to 76) for poor prognosis signatures (adjusted HR=3.63; 95% CI, 0.88 to 15.0; log-rank test, p=0.07). Based on these results, the ongoing MINDACT trial of MammaPrint® was 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 MammaPrint® risk of recurrence classification.

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

Table 6: Results of I-SPY 1: MammaPrint® 70-Gene Signature Results and Trial Outcomes

MammaPrint Risk Category, N (%)	Pathologic Complete Response		Recurrence-Free Survival	
	Proportion of Patients With pCR, % (n/N)	p of Odds Ratio	3-Year RFS, % (n/N)	Hazard Ratio, pCR vs No pCR (95% CI)
Low 11	0% (0/11)	0.00	100% (11/11)	0.00 (–)
High 109	24% (25/105)	0.02	75% (80/105)	0.29 (0.07 to 0.82)

CI: confidence interval; pCR: pathologic complete response; RFS: recurrence-free survival.

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

Other studies comprised primarily small case series and pooled reanalyses of subgroups from previously published retrospective studies. A pooled analysis of 964 patients from previously reported studies with pT1 tumors (≤ 2 cm) included 84% with ER-positive tumors, 68% with HER2-negative tumors (no HER2 information on 23%), 27% with node-positive disease, 68% given no adjuvant treatment, and the rest treated variably. In these patients, overall DMFS at 10 years was 87% (95% CI, 84 to 91) for good prognosis patients and 72% (95% CI, 66 to 78) for poor prognosis patients (HR=2.7; 95% CI, 1.88 to 3.88; $p < 0.001$). Results are confounded by nodal status, HER2 status, and adjuvant therapy.

Kunz et al (2011) conducted a pooled reanalysis of a subgroup of patients' age 35 to 55 years from previously published studies. Patients were 75% ER-positive and 45% node-positive; 60% were untreated, and 40% were treated variably. The 70-gene signature categorized 39% of patients as good prognosis; for these patients, 10-year DMFS was 88% (95% CI, 84 to 92). Bighin et al (2010) commented that nearly 25% of samples from 21 prospectively studied patients were not assessable by the 70-gene signature and that results led to a change in clinical decision in fewer than 20% of cases.

Retel et al (2010) reported a cost-effectiveness analysis that simulated the course of events in a hypothetical cohort of 1000 patients age 50 years who had early operable, node-negative, ER-positive breast cancer, and were treated with 2.5 years of tamoxifen and 2.5 years of an aromatase inhibitor. The authors compared the 70-gene signature with Adjuvant! Online and St. Gallen clinicopathologic classifiers. Although all three strategies were equally clinically effective, St. Gallen was more costly, and the 70-gene signature was most cost-effective when quality-adjusted life-years were taken into account.

Saghatchian et al (2013) evaluated MammaPrint® signatures of frozen tumor samples from patients who had four to nine positive lymph nodes. Approximately half of patients were ER-positive, half were HER2-positive, and half had received adjuvant radiotherapy or chemotherapy. Seventy (40%) of 173 samples were classified as low risk by MammaPrint®, and 103 (60%) were classified as high risk. With median follow-up of 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 et al (2013) investigated the use of MammaPrint® to further risk-stratify 82 ER-negative patients (56% lymph node-negative) who had Oncotype DX® intermediate risk scores. Although MammaPrint® risk classification was significantly associated with 10-year OS in multivariate analysis (log-rank test, p=0.013), this result was confounded by receipt of adjuvant chemotherapy, which also was significantly associated with OS (log-rank test, p=0.024).

To assess the impact of MammaPrint® on treatment decision making, Cusumano et al (2014) distributed clinical information about 194 patients in European countries to multidisciplinary teams in two other countries (e.g., data from the Netherlands was sent to Belgium and Italy) first without and then with MammaPrint® gene signatures. Eighty-six percent of patients were ER-positive, 88% were HER2-negative, and 66% were lymph node-negative. With the addition of MammaPrint® signatures, treatment recommendations changed in 27% of patients, 22% from chemotherapy to no chemotherapy, and 35% from no chemotherapy to chemotherapy. In the subset of 453 ER-positive, HER2-negative patients, treatment advice changed in 32% of patients, with similar proportions changing from chemotherapy to no chemotherapy and vice versa.

Section Summary

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

Blueprint® and TargetPrint®

The Blueprint® molecular subtyping profile was developed using 200 breast cancer specimens that had concordant ER, PR, and HER2 protein levels by IHC and TargetPrint® mRNA readout. Using a three-fold cross validation procedure, 80 genes thought to best discriminate the three molecular subtypes were identified. Blueprint® was confirmed on four independent validation cohorts (total N=784), which included patients from a consecutive series of patients seen at the Netherlands Cancer Institute and treated with adjuvant tamoxifen monotherapy (n=274), a group of patients from the RASTER trial (n=100), and two publicly available data sets (n=410). Additionally, in 133 patients treated with neoadjuvant chemotherapy, the molecular subtyping profile was tested as a predictor of chemotherapy response. The authors concluded that use of Blueprint® classification showed improved distribution of pCR among molecular subgroups compared with local pathology: 56% of patients had a pCR in the basal-type subgroup, 3% in the

MammaPrint® low-risk, luminal-type subgroup, 11% in the MammaPrint® high-risk, luminal-type subgroup, and 50% in the HER2-type subgroup.

Nguyen et al (2012) undertook a comparison of molecular subtyping with Blueprint®, MammaPrint®, and TargetPrint® to locally assess clinical subtyping using IHC and fluorescence FISH. The 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 assays were performed on 132 samples. Concordance between Blueprint® and IHC and FISH testing was 94% for both basal-type and luminal-type subgroups, and 95% for HER2-type. Concordance between Blueprint® and TargetPrint® was 98% for the basal-type, 96% for the luminal-type, and 97% for the HER2-type.

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

Breast Cancer IndexSM (BCI)

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

Ma et al (2008) evaluated MGI along with H/I in 93 patients with lymph node-negative tumors who received adjuvant hormone therapy and found that each index modified the other's predictive performance. High MGI was associated with significantly worse outcome only in patients with high H/I and vice versa. When the H/I ratio and MGI were categorically combined into a single predictor, estimates of 10-year distant metastasis-free survival were 98% (95% CI, 96 to 100), 87% (95% CI, 77 to 99), and 60% (95% CI, 47 to 78) for the low-, intermediate-, and high-risk groups, respectively.

Jerevall et al (2011) combined H/I ratio and MGI into a continuous risk model using 314 ER-positive, node-negative postmenopausal patients from the tamoxifen-only arm of an RCT. The continuous model also was categorized, resulting in proportions of low-, intermediate-, and high-risk patients similar to those reported in the Ma et al 2008 study. This continuous predictor was tested in patients from the no adjuvant treatment arm (n=274) of the same clinical trial, with estimates of rates of distant metastasis at 10 years in the low-, intermediate-, and high-risk

groups of 8.3% (95% CI, 4.7 to 14.4), 22.9% (95% CI, 14.5 to 35.2), and 28.5% (95% CI, 17.9 to 43.6), respectively. 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.

Jankowitz et al (2011) evaluated tumor samples from 265 ER-positive, lymph node-negative, tamoxifen-treated patients from a single academic institution's cancer research registry. BCI categorized 55%, 21%, and 24% of patients as low, intermediate and high risk, respectively, for distant recurrence. Ten-year distant recurrence risk estimates 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 10-year breast cancer-specific mortality risk estimates were 3.8%, 3.6% and 22.1% in low-, intermediate-, and high-risk groups, respectively. In multivariate analysis, BCI was a significant predictor of distant recurrence and breast cancer-specific mortality. In a time-dependent (10-year) ROC curve analysis of recurrence risk, the addition of BCI to Adjuvant! Online risk prediction increased maximum predictive accuracy in all patients from 66% to 76% and in tamoxifen only-treated patients from 65% to 81%.

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

Mammostrat® Breast Cancer Test

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

One study published in 2006 described the development of the assay but provided no information on technical performance (analytic validity). In a validation study in an independent cohort, a multivariate model predicted 50%, 70%, and 87% 5-year DFS for patients classified as high, moderate, and low prognostic risk, respectively, by Mammostrat® ($p < 0.001$). An additional study of the same trial samples used for Oncotype DX® validation (NSABP B-14 and B-20 trials) found that among patients with early, node-negative breast cancer treated only with tamoxifen, those stratified by Mammostrat® into low-, moderate-, and high-risk groups had RFS estimates of 85%, 85%, and 73%, respectively. Both low- and high-risk groups benefited significantly from chemotherapy treatment, but high-risk patients benefited to a greater degree. The moderate-risk group was not well-separated from the low-risk group and thus, moderate-risk results do not appear to provide clinically useful information. A test for an interaction between chemotherapy and the risk group stratification was not significant ($p = 0.13$).

Bartlett et al (2010) used Mammostrat® on 1540 of 1812 patient samples from a consecutive cohort for which minimum nine-year outcomes were available. Tested samples were from tamoxifen-treated patients; 568 of these were from node-negative patients ER-positive tumors who were treated only with tamoxifen. In the latter group, 10-year distant recurrence at 10 years for low-, moderate-, and high-risk patients was 7.6% (95% CI, 4.6 to 10.5), 16.3% (95% CI, 10.0 to 22.6), and 20.9% (95% CI, 12.3 to 29.5) respectively. In multivariate analysis, Mammostrat® was not a significant predictor of RFS in node-negative, ER-positive patients treated only with tamoxifen. However, when all patients (24% node-positive, 20% tumors >2.0 cm, 18% ER-negative, and 46% treated with chemotherapy) with complete Mammostrat® data (n=1300) were included in multivariate analysis, Mammostrat® scores were independent predictors of RFS (p<0.001). In exploratory analyses of various subgroups (e.g., node-negative vs node-positive, ER-negative), Mammostrat® appeared to perform similarly in terms of identifying risk groups. However, patient numbers in these subgroups were small.

BreastOncPx™

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

Tutt et al (2008) published information on the development and validation of the test; no information on analytic validity was provided. To develop a gene signature that was completely prognostic for distant recurrence and not confounded by treatment prediction, samples from untreated patients with early breast cancer were used. The training set (n=142) was derived from a cohort diagnosed with lymph node-negative, stage T1 and T2 breast cancer from 1975 to 1986; ER-positive samples from patients who had had no systemic treatment were selected for analysis. Fourteen genes were eventually selected as most prognostic of time-to-distant metastasis and were given equal weighting in a summary metastasis score (MS). Using a single cut point, 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. Estimated 10-year distant metastasis-free survival was 72% (95% CI, 64% to 78%) for high-risk patients and 96% (95% CI, 90% to 99%) for low-risk patients. 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, HRs for distant MFS and OS 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), respectively. However, between-group differences in risk were 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 with Adjuvant! Online resulted in slightly higher area under the curves (AUCs) for the MS in each case: 0.715 versus 0.661 for distant metastases, and 0.693 versus 0.655 for death. MS was not added to Adjuvant! Online and compared with Adjuvant! Online alone.

NexCourse® Breast IHC4

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

Cuzick et al (2011) evaluated 1125 ER-positive patients from the Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial who did not receive adjuvant chemotherapy, already had an Oncotype DX® Recurrence Score (RS) computed, and had adequate tissue for IHC4 measurements. Of these, 793 (70%) were node-negative, and 59 (5%) were HER2-positive (but were not treated with trastuzumab). A prognostic model that combined the four IHC markers was created (IHC4). In models that combined either IHC4 or Oncotype DX® RS with classical prognostic variables, IHC4 score was found to be similar to Oncotype DX® RS, and little additional prognostic value was seen in the combined use of both scores. In direct comparison, IHC4 score was modestly correlated with Oncotype DX® RS ($r=0.72$); correlation was similar for the subgroup of 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. IHC4 score was validated in a separate cohort of 786 ER-positive women, about half of whom received no endocrine therapy. IHC4 score was significant for recurrence outcomes (HR=4.1; 95% CI, 2.5 to 6.8).

Barton et al (2012) assessed the clinical utility of IHC4 plus clinicopathologic factors (IHC4 + C) by comparison with Adjuvant! Online and the NPI. The study prospectively gathered clinicopathologic data for consecutively treated postmenopausal patients (n=101 evaluable) who had HR-positive, HER2-negative, LN-negative or LN-positive (1-2 nodes), resected early breast cancer. Of 59 patients classified as intermediate-risk by NPI, IHC4 reclassified 24 to low risk and 13 to high risk. IHC4 reclassified 13 of 32 Adjuvant! Online high-risk patients to intermediate risk, and 3 of 32 to low risk. Additionally, 15 of 26 Adjuvant! Online intermediate-risk patients were reclassified to low risk. No Adjuvant! Online low-risk patients were reclassified as high risk.

Prosigna™/PAM50 Breast Cancer Intrinsic Subtype Classifier

The 2014 TEC Assessment reviewed development and validation studies of the PAM50 intrinsic subtype classifier and Prosigna™; these studies are reviewed next. Only 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 1 HER2-associated gene (GRB7) and three proliferation-associated genes (BIRC5 [also called Survivin], MYBL2, CCNB1] that are given special weighting to generate the Prosigna™ recurrence of recurrence (ROR) score. These and other studies published after the 2014 TEC Assessment are reviewed next.

Nielson et al (2014) assessed the analytical performance of Prosigna™ using the proprietary nCounter Analysis System (NanoString Technologies) at NanoString Technologies and two other laboratories. Each tumor sample had been classified by a pathologist as invasive carcinoma (of any type), and all sample testing was blinded. Assay precision was assessed by testing five tumor RNA samples 36 times at the three labs. SD across labs was less than one ROR unit on the

0 to 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 two ROR scores with 95% confidence. Concordance across the three labs for risk categorization in node-negative patients ranged from 88% (95% CI, 73 to 96) to 93% (95% CI, 80 to 98), and in node-positive patients, from 90% (95% CI, 77 to 96) to 95% (95% CI, 84 to 99).

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

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

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

Nielsen et al (2010) compared the PAM50 classifier with standard clinicopathologic factors as represented by Adjuvant! Online and with models based on IHC for biomarkers of intrinsic subtypes. The study used samples from patients diagnosed between 1986 and 1992 with ER-positive, node-negative or node-positive breast cancer at higher-risk (e.g., with lymphovascular

invasion), 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 score that also incorporated the influence of proliferation and tumor size identified patients with a greater than 95% chance of remaining alive and disease-free beyond 10 years. A slightly different gene expression model best fit the node-positive population but did not identify a population at sufficiently low-risk that adjuvant hormone therapy would likely be considered sufficient.

Because the cohort used to generate the models evaluated in this study was biased toward higher-risk early breast cancers, it is likely not generalizable. Nor did the authors clearly identify a final model for clinical use. Rather, the authors outlined potential additional studies.

Cheang et al (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 relapse-free survival (RFS; $p < 0.001$) and OS ($p < 0.001$). 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 OS differences exceeding 20%. There was a less than 2% difference for non-HER2-enriched tumors (interaction test, $p = 0.03$ for RFS and 0.03 for OS). Within clinically defined HER2-positive tumors, 79% (72/91) 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%, $p < 0.001$). There was no significant difference in benefit from CEF versus CMF in basal-like tumors.

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

BreastPRSTTM

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

D'Alfonso et al (2013) sought to translate a previously published validation study of BreastPRS, using fresh-frozen tissue, to FFPE tumor samples. The authors compared BreastPRSTTM to Oncotype DX® and correlated recurrence scores with clinicopathologic features. A linear relationship of BreastPRSTTM prognostic scores between fresh-frozen and FFPE formats was observed. Using publically available whole genome profiles from a series of untreated ER-

positive, node negative patients, investigators assessed the ability of BreastPRSTTM to reclassify Oncotype DX[®] intermediate-risk patients into high- or low-risk categories with clinically significant differences in outcomes. BreastPRSTTM prognosis scores were compared with Oncotype DX[®] recurrence scores in 246 patients with invasive breast carcinoma and known Oncotype DX[®] results. Using this series, a 120-gene Oncotype DX[®] approximation algorithm to predict Oncotype DX[®] risk groups was then applied to a series of untreated, ER-positive, node-negative patients from previously published studies with known clinical outcomes. Of 30 high-risk Oncotype DX[®] cases, 27 (90%) were classified as high-risk by BreastPRSTTM, and 95 low-risk Oncotype DX[®] cases (76%) were classified as low-risk by BreastPRSTTM. The correlation of recurrence score and risk group between Oncotype DX[®] and BreastPRSTTM was statistically significant ($p < 0.001$). Fifty-nine (23%) of 260 patients from four previously published studies were classified as intermediate-risk when the 120-gene Oncotype DX[®] approximation algorithm was applied. BreastPRSTTM reclassified the 59 patients into binary risk groups (high vs low risk), with 23 (39%) patients classified as low risk and 36 (61%) as high risk (HR for a high-risk classification, 3.64; 95% CI, 1.40 to 9.50; $p = 0.029$). Ten-year RFS was 90% in the low-risk group and 60% in the high-risk group. The authors concluded that BreastPRSTTM prognosis score is comparable with Oncotype DX[®] recurrence score and can reclassify Oncotype DX[®] intermediate-risk patients into two groups with clinically significant differences in RFS.

EndoPredictTM

Varga et al (2013) analyzed the EndoPredictTM (EP) test in 34 hormone positive, invasive breast cancer cases and compared the EndoPredict (EP) scores with the Oncotype DX[®] recurrence scores (RS) obtained from the same cancer samples. EP classified 11 patients as low risk and 23 patients as high risk, whereas RS classified 15 patients as low risk, 10 patients as intermediate risk, and nine patients as high risk. There were major discrepancies in 6 of 34 cases (18%), with low-risk RS classified as high risk by EP in 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.

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

Dubsky et al (2013) examined predictive ability of EP and EPclin for early (0-5 years) and late (>5 years postdiagnosis) disease recurrence. Tumor samples from chemotherapy-untreated, ER-positive, HER2-negative patients who participated in one of two RCTs (ABCSG6 or ABCSG8)

were assayed (total N=1702). In the trials, patients received either tamoxifen for five years or tamoxifen for two years followed by anastrozole for three years. Forty-nine percent (n=832) of patients were classified as low risk by EP score, and 51% (n=870) were classified as high risk. Only relative estimates (i.e., HRs) of distant recurrence were reported. In comparison with low-risk patients, high-risk patients had an almost three-fold increase in the risk of recurrence in the first five years after diagnosis (HR=2.80; 95% CI, 1.81 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, 1066 (63%) of 1702 patients were classified as low risk, and 636 (37%) were classified as high risk. In comparison with low-risk patients, high-risk patients had an almost five-fold risk of recurrence within the first five years (HR=4.82; 95% CI, 3.12 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). Given the discrepancy in risk classification between EP and EPclin, and the incomplete reporting of recurrence and survival outcomes by risk groups, this evidence is insufficient to demonstrate improved prognostic accuracy for individual patients with EndoPredict.

Test Comparison Studies

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

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

Hornberger et al (2012) conducted a systematic review on the clinical validity, clinical utility, change in clinical practice, and economic implications of early-stage breast cancer stratifiers. Fifty-six articles published original evidence addressing the 21-gene recurrence score (Oncotype DX®) (n=31), 70-gene signature (MammaPrint®) (n=14), Adjuvant! Online (n=12), 5-antibody

immunohistochemistry panel (Mammostrat®) (n=3), and 14-gene signature (BreastOncPx™) (n=1). Oncotype Dx® recurrence score satisfied Level 1 evidence for estimating distant recurrence risk (DRR), OS, and response to adjuvant chemotherapy, and Level 2 evidence for estimating local recurrence risk. Mammostrat® and MammaPrint® satisfied Level 2 evidence for estimating DRR and OS. Adjuvant! Online satisfied Level 2 evidence for estimating DRR, OS, and chemotherapy response. BreastOncPx™ satisfied Level 3 evidence for predicting DRR and OS. Ten studies reported changes in clinical practice patterns using Oncotype DX®; overall, Oncotype DX® was associated with change in treatment recommendations and/or decisions in 21% to 74% of cases.

Fan et al (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. The classifiers included the 21-gene recurrence score, the 70-gene signature, the H/I ratio, and the intrinsic subtype classifier (similar to the commercially available PAM50). Most highly correlated were the 21-gene recurrence score and the 70-gene signature, with a Cramer V of 0.6 (scale 0 to 1 with 1 indicating perfect agreement). More specifically, 81 (79%) of 103 samples with a recurrence score of low or intermediate risk were classified as having a low-risk 70-gene profile. Restricting the analysis to 225 ER-positive samples slightly reduced the correlation. Analysis was not further restricted to node-negative patients, the present indication for both tests.

Espinosa et al (2005) compared the 21-gene recurrence score (Oncotype DX®), the 70-gene signature (MammaPrint®), and the 2-gene ratio (H/I ratio) in 153 patients with ER-positive breast cancer treated with adjuvant tamoxifen. Sixty-two percent of patients were node-negative, and 63% were additionally treated with chemotherapy. Estimated distant metastasis-free survival for recurrence score risk groups was 98% for low-risk, 81% for intermediate-risk, and 69% for high-risk patients; for the 70-gene signature, estimates were 95% for good prognosis and 66% for poor prognosis patients; and for the 2-gene ratio, estimates were 86% for favorable and 70% for unfavorable prognosis. Correlation between the 21-gene recurrence score and the 70-gene signature was good (Cramer V=0.6). Slightly more variation in distant metastasis-free survival was explained by the combination of the 21-gene recurrence score and either Adjuvant! Online (25.8 ± 1.4) or the Nottingham Prognostic Index (NPI; 23.7 ± 1.5) than by the combination of the 70-gene signature with Adjuvant! Online (23.1 ± 1.2) or the NPI (22.4 ± 1.3), but differences were small and any combination was significantly better than any test or clinicopathologic classifier alone.

Two recent papers compared Oncotype DX® and other gene expression profiles. Kelly et al (2012) evaluated Oncotype DX® and PAM50 in 108 cases and found good agreement between the 2 assays for high- and low-prognostic risk assignment; PAM50 assigned about half of Oncotype DX® intermediate-risk patients to the PAM50 luminal A (low risk) category. Prat et al (2012) evaluated several gene expression tests, including Oncotype DX®, PAM50, and MammaPrint®, in 594 cases and found all predictors were significantly correlated (Pearson *r* range, 0.36-0.79; $p < 0.001$ for each comparison).

Additional Applications

Based on a study published in May 2008 that compared Oncotype DX® ER and PR results with traditional IHC results, Genomic Health is now including quantitative ER and PR component

results in Oncotype DX® 21-gene profile reports. The study reported 90% or better concordance between the two assays, but quantitative ER by Oncotype DX® was more strongly associated with disease recurrence than IHC results. However, ER and PR analysis is traditionally conducted during pathology examination of all breast cancer biopsies, whereas Oncotype DX® is indicated only for known ER-positive tumors, after the pathology examination is complete, the patient meets specific criteria, and patient and physician are considering preferences for risk and chemotherapy. Thus, Oncotype DX® should not be ordered as a substitute for ER and PR IHC. Additionally, accepted guidelines for ER and PR testing outline standards for high-quality IHC testing and do not recommend confirmatory testing; thus the 21-gene RS should not be ordered to confirm ER/PR IHC results. Similarly, guidelines for HER2 testing specify IHC and/or FISH methods. In one large study, the HER2 component of the 21-gene assay was shown to strongly correlate with FISH results, but another study noted significant discrepancies. As a result, and without evaluation and support from guidelines, it has been recommended that the 21-gene assay not be ordered to determine or confirm HER2.

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

Clinical Input Received through Physician Specialty Societies and Academic Medical Centers

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

Summary

21-Gene Recurrence Score (Oncotype DX®): The assay is supported by strong evidence of clinical validity, i.e., that the recurrence score (RS) is strongly associated with risk of distant recurrence in women with invasive breast cancer that is positive for hormone receptors, negative for HER2, and without lymph node involvement. Limited but sufficient evidence supports analytic validity and clinical utility in this population. Oncotype DX® adds additional risk information to conventional clinical classification of individual high-risk patients and identifies a subset of patients who would otherwise be recommended for chemotherapy but who are actually at lower risk of recurrence (average 7% to 9% risk at 10 years; upper 95% confidence interval 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.

In similar women who are node-positive, evidence is less clear that the risk of recurrence in low-risk RS patients is sufficiently low or that the benefit of chemotherapy is insufficiently large, to recommend avoiding otherwise currently recommended treatment. Additional studies are necessary and ongoing.

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

70-Gene Signature (MammaPrint®)

A large number of studies of clinical validity, and a few attempting to address the clinical utility of the 70-gene signature have been published. Several studies have pooled and reanalyzed subsets of previously published data in attempts to arrive at more homogeneous patient samples. Nevertheless, MammaPrint® studies continue to suffer from confounding due to heterogeneous patient samples. Pooled reanalyses of subpopulations may control for one variable (e.g., nodal status), but confounding remains from other variables (e.g., treatment heterogeneity). Results for MammaPrint® good prognosis patients have confidence intervals that extend into ranges that likely confer too much risk for U.S. patients and providers. Because the test result is not a continuous numerical result, patients cannot view their result within the spectrum of good prognosis results and adjust their preferences accordingly. The recently published Microarray Prognostics in Breast Cancer (RASTER) study represents an improved study design; results suggested that MammaPrint® may accurately re-classify early, node-negative invasive breast cancer patients classified as high risk by clinical and pathologic variables to low risk, such that chemotherapy may not be necessary. However, patient numbers and events were too low to make firm conclusions, and follow-up is not yet sufficiently mature.

BluePrint® and TargetPrint®

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

Breast Cancer IndexSM, Mammostrat® Breast Cancer Test, BreastOncPx™, NexCourse® Breast IHC4, Prosigna™, BreastPRS™, EndoPredict™

Evidence supporting these tests comprises clinical validity data showing that the test is independently and significantly associated with distant recurrence and that the test can identify a lower risk population of women with early, invasive breast cancer who may not need chemotherapy. In almost all cases, the test is not added to and compared with a standard clinicopathologic classifier such as Adjuvant! Online. The BreastOncPx™ validation study included a ROC analysis comparing the test with Adjuvant! Online, but no clear evidence supporting clinical utility was available. NexCourse® Breast IHC4 (immunohistochemical markers) was compared with standard clinicopathological prognostic classifiers in a reclassification analysis and was shown to accurately reclassify significant numbers of patients from high and intermediate risk to low risk, but sample size was small and insufficient for conclusions.

Practice Guidelines and Position Statements

National Comprehensive Cancer Network

Current National Comprehensive Cancer Network (NCCN) guidelines for breast cancer (version 3.2014) do not include any gene expression signature tests. However, pending results of prospective RCTs for Oncotype DX® (TAILORx) and MammaPrint® (MINDACT), Oncotype DX® may be considered an option when evaluating patients who have invasive breast cancer with all of the following features: (category 2A recommendation)

- hormone receptor-positive;
- HER2-negative;
- node-negative OR not greater than 2 mm axillary node metastasis; AND
- tumor size 0.6 to 1.0 cm with unfavorable features OR larger than 1 cm.

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

American Society of Clinical Oncology

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

St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer

The 2013 St. Gallen expert panel emphasized the identification of intrinsic breast cancer subtype when deciding whether to use systemic adjuvant chemotherapy. Panelists did not recommend any particular assay for this purpose. For patients with lymph node–negative luminal (HR-positive, HER2-negative) disease, Oncotype DX® and MammaPrint® were considered to provide “accurate and reproducible prognostic information.” Oncotype DX® also was considered predictive of response to chemotherapy. No consensus recommendation was made for lymph node-positive disease. Molecular diagnostic testing for patients with lymph node–negative, ER-positive, HER2-negative disease and low clinical risk (e.g., tumor size ≤1 cm) was considered unnecessary; similarly, in patients at high risk due to clinicopathologic factors (e.g., tumor size >5 cm, inflammatory breast cancer, ≥4 involved lymph nodes, or a very low ER positivity [e.g., <5%]), molecular diagnostic testing was considered unnecessary.

U.S. Preventive Services Task Force

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

Key Words:

Microchip array, tumor gene expression, breast cancer, Oncotype test, Oncotype™ DX, MammaPrint®, Breast Cancer Gene Expression Ratio, Mammostrat, THEROS Breast Cancer Index, 12 gene expression test, colon cancer, BreastOncPx, PAM50 Breast Cancer Intrinsic Classifier, Geneoptix, NexCourse, TargetPrint, Blueprint, Prosigna Breast Cancer Assay

Approved by Governing Bodies:

MammaPrint® was U.S. Food and Drug Association (FDA)-approved on February 6, 2007. MammaPrint® is performed in Agendia laboratories in the Netherlands and in California.

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

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

Benefit Application:

Coverage is subject to member's specific benefits. Group specific policy will supersede this policy when applicable.

ITS: Home Policy provisions apply

FEP contracts: FEP does not consider investigational if FDA approved and will be reviewed for medical necessity.

Coding:

CPT codes:

81479	Unlisted molecular pathology procedure
81599	Unlisted multianalyte assay with algorithmic analysis
84999	Unlisted chemistry procedure

The following code is for use with the Prosigna Breast Cancer Assay:

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 (effective 07/01/2014)
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HCPCS codes:

S3854	Gene expression profiling panel for use in the management of breast cancer treatment
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Policy History:

Medical Policy Group, June 2004 (4)
 Medical Policy Administration Committee, July 2004
 Available for comment August 11-September 24, 2004
 Medical Policy Group, July 2005 (1)
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 Available for comment January 28-March 13, 2006
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 Medical Policy Group, July 2007 (4)
 Medical Policy Group, February 2008 (2)
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 Medical Policy Group, February 2009 (3)
 Medical Policy Administration Committee, February 2009

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 Medical Policy Group, December 2009 (1)
 Medical Policy Administration Committee, December 2009
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 Medical Policy Group, June, 2011 (1): Update to Description, Key Points Coding and References; Added tests BreastOncPx and PAM50 to Policy as investigational and to Key Words
 Medical Policy Administration Committee, August 2011
 Available for comment August 11 – September 26, 2011
 Medical Policy Panel, December 2012
 Medical Policy Group, January 2013 (1): Update to Descriptions, Policy, Key Points, Key Words, and References related to addition of bilateral disease as investigational Oncotype statement; addition of newer assay considered investigational and addition of Oncotype for DCIS as investigational; removed and archived policy statement prior to December 2009.
 Medical Policy Administration Committee, February 2013
 Available for comment February 21 through April 7, 2013
 Medical Policy Group, July 2013 (1): Update to Policy section with clarifying (included, but not limited to) phrase added to ‘use of other gene assays’; update to Key Words with addition of TargetPrint and Blueprint gene assays; update to Coding section with addition of 81479 and 81599 to policy.
 Medical Policy Administration Committee, July 2013
 Medical Policy Group, May 2014 (1): Added new code 0008M related to Prosigna Breast Cancer Assay, which is a non-covered and investigational indication; effective 07/01/14
Medical Policy Panel, July 2014
Medical Policy Group, July 2014 (1): Update to Policy, Key Points and References with addition of noncoverage statements related to breast cancer in men and new tests Blueprint and TargetPrint; removed the “non-fixed” verbiage from criteria for unilateral tumor related to Oncotype Dx
Medical Policy Administration Committee, August 2014
Available for comment August 8 through September 22, 2014

This medical policy is not an authorization, certification, explanation of benefits, or a contract. Eligibility and benefits are determined on a case-by-case basis according to the terms of the member's plan in effect as of the date services are rendered. All medical policies are based on (i) research of current medical literature and (ii) review of common medical practices in the treatment and diagnosis of disease as of the date hereof. Physicians and other providers are solely responsible for all aspects of medical care and treatment, including the type, quality, and levels of care and treatment.

This policy is intended to be used for adjudication of claims (including pre-admission certification, pre-determinations, and pre-procedure review) in Blue Cross and Blue Shield's administration of plan contracts.