



Cigna Medical Coverage Policy

Subject Genetic Expression Assays for Breast Cancer Prognosis

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Coverage Policy

Cigna covers Oncotype DX™ Breast Cancer Assay as medically necessary to assess the need for adjuvant chemotherapy in women with recently diagnosed breast cancer when ALL of the following criteria are met:

- Breast tumor is stage 1 or stage 2.
- Individual is axillary-node negative or has axillary-node micrometastasis no greater than 2.0 millimeters.
- There is no evidence of distant metastatic breast cancer.
- Breast tumor is estrogen-receptor positive.
- Breast tumor is HER2-receptor negative.
- Individual is a candidate for possible adjuvant chemotherapy (i.e., chemotherapy is not precluded due to other factors), and testing is being done specifically to guide the decision as to whether or not adjuvant chemotherapy will be used.

Cigna does not cover Oncotype DX Breast Cancer Assay for ANY other clinical evaluation because it is considered experimental, investigational or unproven.

Cigna does not cover ANY of the following assays of genetic expression in breast tumor tissue, because each is considered experimental, investigational or unproven:

- Oncotype DX Breast Cancer Assay for DCIS
 - Breast Cancer Gene Expression Ratio
 - HERmark[®] Breast Cancer Assay
 - MammaPrint[®]
 - Rotterdam Signature 76-Panel
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General Background

Breast cancer is a malignant tumor that originates in the breast cells of both males and females. If an individual is suspect for breast cancer, needle aspiration and/or a biopsy may be performed. Tissue obtained from a biopsy may be tested by a hormone receptor assay to determine the presence or absence of estrogen (i.e., estrogen-receptor- [ER] positive or ER-negative) and progesterone (i.e., progesterone [PR]-positive or PR-negative), and for the presence of the human epidermal growth factor receptor 2 [HER2], also called HER2/neu, epidermal growth factor receptor 2 (EGFR2), and erbB2.

The American Joint Committee on Cancer (AJCC) staging system provides a strategy for grouping patients with respect to prognosis. Therapeutic decisions are formulated in part according to staging categories but primarily according to tumor size, lymph node status, estrogen-receptor and progesterone-receptor levels in the tumor tissue, human epidermal growth factor receptor 2 (HER2/neu) status, menopausal status, and the general health of the patient (National Cancer Institute [NCI], 2013). Prognostic factors in a male include the size of the tumor and lymph node involvement. The American Cancer Society ([ACS], 2013) and the NCI (2013) recommend testing for estrogen- and progesterone-receptor status in men. Additionally, a small number of breast cancers in men may express the HER2/neu protein.

Currently, clinicopathologic and immunohistochemistry markers and algorithm tools are used to assist in predicting the 10-year disease-free (DFS) and overall survival (OS) of breast cancer patients based upon prognostic factors. These predictions are taken into consideration during patient management in order to offer the optimal treatment pathways, including the use of chemotherapy. However, considerable differences exist regarding the selection of women who should be treated with adjuvant chemotherapy. It is suspected that some patients with ER-positive, lymph node-negative (N0) breast cancer receive chemotherapy without clear benefit, leading to potential over-treatment, while others destined to experience recurrence are not treated. Better prognostic tools are needed to help determine optimal treatment options for patients with early-stage breast cancer (Andre and Puztai, 2006; Bogaerts, et al., 2006; Kaklamani, 2006; Lyman and Kuderer, 2006).

Assays of genetic expression, gene expression analyses, or gene-expression profiling have been proposed as an adjuvant tool to assist in determining OS, recurrence probability, appropriate treatment options, and responsiveness to chemotherapy. Used in conjunction with consensus guidelines and risk assessments, gene profiling assays may help to identify those women who do not need adjuvant chemotherapy (National Comprehensive Cancer Network[®], [NCCN[®]], 2013; ECRI, 2011; Paik, 2006).

Gene Expression Testing Platforms

The four conventional platforms utilized for genetic expression profiling include immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), polymerase chain reaction (PCR), and high resolution karyotyping (G-Banding). IHC involves designing monoclonal antibodies that bind to the molecule being assessed. Formalin-fixed paraffin-embedded tissue is then stained with the antibodies and the expression of the protein is assessed under a microscope. FISH is an established technique that labels specific regions of deoxyribonucleic acid (DNA), using sequence specific oligonucleotides (i.e., short sequences of DNA) to identify chromosomal deletions, additions or rearrangements. Because FISH uses individual probes, it reveals DNA aberrations of only the probe-targeted segments. IHC and FISH are the two established platforms used for evaluating HER2 levels in breast cancer patients. PCR is an established laboratory method used to make numerous copies of a specific DNA sequence, utilizing pairs of oligonucleotide primers to replicate and alternate rounds of DNA. Real-time polymerase chain reaction, also called quantitative real time polymerase chain reaction (Q-PCR/qPCR/qrt-PCR) or kinetic polymerase chain reaction (KPCR), is a PCR technology used to simultaneously amplify and quantify

the targeted DNA molecule. In reverse transcriptase PCR (RT-PCR) an RNA strand is reverse transcribed into its DNA complement (cDNA). When high resolution G-banding is used, chromosomes are first treated with trypsin, an enzyme that degrades proteins. The chromosomes are then stained with Giemsa which produces a banding pattern of light and dark stripes enabling identification of each chromosome. Methylation-specific PCR (MSP) assesses the methylation status of DNA (American Association of Clinical Chemistry [AACC], 2010; Kibel and Reiter, 2007). (American Association of Clinical Chemistry [AACC], 2010; Turaga, et al., 2010; National Academy of Clinical Biochemistry [NACB], 2009a; NACB 2009b; Kibel and Reiter, 2007).

Utilization of the four platforms has allowed development of various genetic expression assays for breast cancer. Some assays are performed in a centralized Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory which means the test does not require U.S. Food and Drug Administration (FDA) approval. Various companies are seeking FDA approval for assays, which allows distribution and use by multiple laboratories. These assays are not advocated as stand-alone tools. They are proposed as an adjuvant tool to be used with other recognized prognostic indicators. The Oncotype DX™ 21-gene breast cancer assay (Genomic Health, Redwood City, CA) is recommended by the American Society of Clinical Oncologists (ASCO) (2007) and NCCN (2013) for use in a specific subgroup of women with breast cancer. The MammaPrint® 70-gene assay (Agendia USA, Irvine, CA, formerly Molecular Profiling Institute [MPI] Inc., Phoenix, AZ; and Agendia BV, Amsterdam, The Netherlands) has received FDA approval for specific indications in women with breast cancer. Numerous other assays such as the Breast Cancer Gene Expression Ratio (i.e., H/I™) (AviaraDx, Inc., Carlsbad, CA), HERmark Breast Cancer Assay (Monogram Biosciences, South San Francisco, CA), and the Rotterdam Signature 76-gene panel (Veridex LLC, a Johnson & Johnson Co, Warren, NJ) have also been proposed for use.

Oncotype DX Breast Cancer Assay: The test is recommended for use after the original breast cancer surgery and is proposed for newly diagnosed patients with node-negative or node-positive, ER-positive, HER2-negative invasive breast cancer (Genomic Health, 2004-2013). The purpose of Oncotype DX Breast Cancer Assay is to quantify the likelihood of distant recurrence (i.e., within 10 years) in a woman with breast cancer, and is used as one factor in determining whether or not a patient is a candidate for chemotherapy. Using tumor tissue, ribonucleic acid (RNA) is extracted, purified and analyzed for expression of a panel of 21 genes using quantitative reverse transcription polymerase chain reaction (RT-PCR) on formalin-fixed, paraffin-embedded tumor tissue. A Recurrence Score™ (RS) is calculated from the gene expression results using a proprietary Oncotype DX algorithm. The RS is based on a scale of 0–100. A score of less than 18 is considered low-risk; 18–31 is intermediate-risk; and a score over 31 is designated as high-risk. Each RS correlates with a specific likelihood of distant recurrence at 10 years. This assay is not proposed for or used as a test to monitor the response of a specific chemotherapy drug.

Literature Review

The published peer-reviewed literature supports the accuracy and clinical utility of Oncotype in its ability to predict the benefits of chemotherapy in women with localized stage 1 or stage 2 breast cancer who are ER-positive and HER2-receptor negative with no evidence of metastasis. The patient should also be axillary-node-negative which includes micrometastasis no greater than 2.0 millimeters. Tissue samples from tumor banks were used to validate the prognostic ability of the 21-gene assay. Studies reported that patients with a low recurrence score (RS) had a 10-year distant recurrence-free survival rate of 93.2%; 85.7% for patients with an intermediate RS, and 69.5% for patients with a high RS (Tang, et al., 2011; Lo, et al., 2010; Mamounas, et al., 2010; Toi, et al., 2010; Chang, et al., 2008; Habel, et al., 2006; Paik, et al., Aug 2006; Cobleigh, et al., 2005; Gianni, et al., 2005; Paid, et al., 2004). Supporting data on the use of Oncotype in men and the value of repeat assays after the initial assessment are lacking.

Most recently Genomic Health has expanded the criteria for Oncotype to include postmenopausal women, hormone-receptor positive, with node-positive (1–3 nodes) breast cancer. The prognostic value of this assay is also being studied in women with loco-regional recurrence and disease free survival at five-years. However, at present evidence in the published peer-reviewed literature is limited to small prospective observational and retrospective subgroup analyses of small patient cohorts from previously published randomized controlled trials (RCTs) (Eirman, 2012; Solin, 2012; Dowsett, 2010). There is insufficient high level evidence to support clinical utility and improved health outcomes of Oncotype in this subset of breast cancer patients. Recent clinical trials have also evaluated the accuracy of HER2 status assessed by Oncotype using RT-PCR compared to IHC and/or FISH reporting as high as a 50% false-negative rate with Oncotype.

Eirmann et al. (2012) reported results of a prospective study to evaluate the impact of Oncotype DX on adjuvant decision-making in a cohort of consecutive patients with ER+, HER2-negative early breast cancer (EBC). Secondary study objectives assessed the impact of the RS on patients' decisional conflict, on physicians' confidence in their treatment recommendations, to evaluate the rate of therapy actually administered in relation to recommended therapies, and to assess the pharmaco-economic impact of RS-guided adjuvant decision-making for German clinical practice. Eligible female patients presented with operable EBC, ER positive, HER2 negative by IHC or FISH, tumor size of ≥ 1 cm (i.e., T1, 2, 3 excluding those with dermal involvement) or < 1 cm if at least one histological unfavorable characteristic (i.e., high histological grade, angiolymphatic invasion and p-53 positive), node-negative or histologically verified lymph node metastases in up to three lymph nodes. Further inclusion criteria were age ≥ 18 years, good performance status (ECOG 0–1, Karnofsky Index ≥ 70) and no contraindication for receiving systemic chemoendocrine therapy.

There were 366 assessable patients; 244 were node-negative (67%), and 122 node-positive (i.e., 1-3 nodes, 33%). After the recurrence score (RS) was known the initial treatment recommendation was revised in 33% of all assessable patients (30% in node-negative (N0) and 39% in node-positive (N+) subgroups). Overall, physicians' confidence increased in 45% of N0 patients and 46% of N+ cases ($P < 0.001$). Completed questionnaires were available for 325 patients. The difference from the pre- to post-test decision conflict score (DCS) was statistically significant for all patients (6%, $P = 0.028$) and for patients with low RS values (11%, $P = 0.003$). Treatment administered differed from the post-RS recommendation in 45 cases (9% of patients with N0, and 20% with N+ disease). There was an overall 19% net reduction in adjuvant chemotherapy usage when the actual number of chemotherapies given was compared with the initial treatment recommendation regardless of the nature of the pre-test recommendation. Data suggest that there is significant influence relative to knowledge of the RS and resulting treatment recommendations; however, disease outcome was not correlated with RS results and therefore no conclusions can be made regarding effect on net health outcomes for patients with either N0 or N+ disease.

Dabbs et al. (2011) conducted a quality assurance study on tumor specimens ($n=843$) from patients who were tested for HER2 status with Oncotype compared to HER2 status as determined by IHC and/or FISH at three institutions. Using IHC/FISH, 784 specimens were classified as negative, 36 as positive and 23 as equivocal. Of the 784 negative cases, 779 (99%) were classified as negative by the Oncotype RT-PCR assay. Of the 36 positive cases, Oncotype reported 10 as positive, 12 as equivocal, and 14 as negative. Oncotype reported all 23 equivocal cases as negative. The false-negative rate for Oncotype for HER2 was $> 50\%$. With the exception of one case, none of the clinically significant discordant patient cases contained more than 50% invasive carcinoma in the tissue block sent for Oncotype testing, suggesting dilution of tumor mRNA by nontumor tissue.

Hornsberger et al. (2012) conducted a systematic review of the literature assessing clinical validity/utility, change in clinical practice, and economic implications of the following early stage breast cancer stratifiers: 21-gene recurrence score (Oncotype DX), 70-gene signature (MammaPrint), 5-gene expression index (Molecular Grade Index, bioTheranostics, Dan Diego, CA), 5-antibody immunohistochemistry (IHC) panel (Mammostrat, Clariant, Alisa Viejo, CA), and the 14-gene signature (BreastOncPx, US Labs, Irvine, CA). Clinical validity was defined as the ability of the assay to predict the clinical endpoint(s) of interest. Clinical utility was defined as the balance of associated benefits and risks if the assay is introduced into clinical practice. The primary objective was to systematically grade the Level-of-Evidence (LOE) of the eligible studies. The secondary objective was to document studies that provide evidence on changes in practice patterns and health economic implications of the stratifiers.

Studies were graded as Category A, representing prospective, randomized clinical trial designs, Category B: prospective studies using archived tissue samples, Category C: prospective, observational registry studies in which treatment and follow-up are not dictated, and Category D representing retrospective/observational studies. The authors note "Category B was included in this framework because a positive result from this type of study is less likely to be a 'play of chance' than a similar result from a Category C, prospective, observational registry." According to the authors, no eligible study met the Category A classification.

The authors also graded evidence as Level I if there was at least one validation study from Category A, or one or more validation studies from Category B with consistent results. Level II evidence includes at least one Category B study or two or more studies from Category C. Level III evidence includes at least one study from Category C and Levels IV and V includes studies from Category D.

Fifty-six articles were included in the review including the 21-gene recurrence score (n = 31, 14 related to prognosis or prediction), 70-gene signature (n = 14, 11 related to prognosis or prediction), Adjuvant! Online (n = 12, 5 related to prognosis or prediction), 5-antibody immunohistochemistry panel (n = 3, all related to prognosis or prediction), 5-gene expression index (n = 1, related to prognosis or prediction), and 14-gene signature (n = 1, related to prognosis or prediction). According to the review, there was Level 1 evidence that the 21-gene recurrence score estimated distant recurrence risk (DRR), overall survival (OS), and response to adjuvant chemotherapy, and Level II evidence estimating local recurrence risk. There was Level II evidence that the 5-antibody immunohistochemistry panel and 70-gene signature estimated DRR and OS. There was Level II evidence that Adjuvant! Online, a software model that predicts the benefit of adjuvant therapy, estimated DRR, OS, and chemotherapy response. The 5-gene expression index satisfied Level III evidence for predicting DRR and the 14-gene signature satisfied Level III evidence for predicting DRR and OS. No recommendations were made regarding the application of these assays into routine clinical practice.

Solin et al. (2012) retrospectively evaluated 388 tumor samples to assess the significance of biologic subtype and 21-gene recurrence score relative to local recurrence and local–regional recurrence after breast conservation treatment with radiation. Specimens were taken from the Eastern Cooperative Oncology Group E2197 randomized controlled trial (RCT) that compared two adjuvant systemic chemotherapy regimens, doxorubicin plus cyclophosphamide (AC) versus doxorubicin plus docetaxel (AT). Patients had operable breast cancer, 0–3 positive lymph nodes and tumor size > 1.0 centimeters. The subset for this study was patients with known 21-gene recurrence scores (RS) and treated with surgery, systemic chemotherapy, and definitive radiation treatment. Follow-up ranged from 3.7–11.6 years (median, 9.7 years). Ninety patients had one positive node, 41 patients had two positive nodes and 16 patients had three positive nodes.

Neither biologic subtype nor the RS was associated with local recurrence or local–regional recurrence on univariate analyses ($p > 0.12$, each). The 10-year local recurrence rates were 3.1%, 2.9% and 7.6 % for low, intermediate, and high RS, respectively ($p = 0.24$). Analysis of the RS as a continuous variable, restricted to HR-positive tumors yielded a borderline statistically significant hazard ratio for local recurrence ($p = 0.07$) and a statistically significant hazard ratio for local–regional recurrence ($p = 0.03$). No significant differences were seen when the RS was combined with patient age ($p \geq 0.09$), HR positive tumors ($p \geq 0.02$), or HER2-negative tumors ($p \geq 0.02$). Analysis of RS combined with HR positive, HER2-negative tumors showed a significant result for local and local-regional recurrence when adjusted for age ($p \leq 0.03$). Multivariate analyses for local recurrence and local–regional recurrence using the variables of chemotherapy arm, patient age, HR status, pathologic axillary lymph node status, histologic grade, pathologic T stage, biologic subtype, and RS identified no statistically significant variables ($p \geq 0.26$, each). Subgroups identified with 10-year recurrence > 10% were 1) HR-positive tumors with a high RS; 2) HR positive, HER2-negative with a high RS; and 3) patients age ≤ 39 years. However, the authors cautioned that the results in these subgroups were based on a small number of patients with wide 95% confidence intervals. In this study, the rates of local recurrence and local–regional recurrence were higher with increasing RS but were not statistically significant. Results related to number of positive nodes were not reported. Author-noted limitations include: patients treated in this study predated the era of adjuvant trastuzumab for HER2-positive tumors (15% in this study), the biologic subtype was approximated, and the study represented a subset of the overall population in the original E2197 study. Additional study limitations include retrospective design and limited data regarding impact on patient management.

Dvorak et al. (2012) compared the HER2 status documented on Oncotype reports (n=194) to the HER2 results conducted by fluorescence in situ hybridization (FISH) in the same patients to determine the frequency of discrepancy between HER2 status performed by these two tests and to characterize the clinicopathologic features of discrepant cases. Image analysis was performed on cases with discrepancies in the HER2 results. Overall agreement was 96%, negative agreement was 100%, and percent positive agreement was 50%. Three of eight (38%) discrepant cases showed heterogeneous amplification by FISH. Seven of eight (88%) cases had < 50% invasive tumor in the Oncotype DX tissue block. In three of eight discrepant cases, the blocks used for FISH and those sent for Oncotype DX testing were different. Seven discrepant cases had < 50% of the surface area involved by invasive cancer.

Dowsett et al. (2010) evaluated 1231 tissue samples from a previously reported randomized controlled trial to determine the prognostic value of Oncotype for distant recurrence in hormone receptor-positive postmenopausal women with localized node-negative (N0) (n=872) or node-positive (N+) (n=306) breast cancer patients who were treated with either tamoxifen (n=609) or anastrozole (n=622). The samples were obtained from the tamoxifen and anastrozole arms of the Arimidex, Tamoxifen, Alone or in Combination (ATAC) Trial (n=4160)

which evaluated the safety and efficacy of five years of anastrozole, tamoxifen, or both in postmenopausal women. Sixty-three patients had $\geq 4+$ nodes and 243 patients had 1–3 positive nodes. Node status was unknown in the remaining patients. Tumor sizes included ≤ 2 centimeters (cm) (67%), 2–5 cm (31%), > 5 cm (1.5%), and unknown (0.3%). In the N0 group, 432 women received tamoxifen and 440 received anastrozole. In the N+ group, 152 women received tamoxifen and 154 received anastrozole. The median follow-up was 8.5 years. Tumor size and the Oncotype Recurrence Score (RS) were each separately statistically significant ($p < 0.001$ each) in predicting time to distant recurrence (TTDR) in node-negative (N0) patients. The RS was also predictive of TTDR in node positive (N+) patients ($p = 0.002$) in multivariate analyses. The number of positive nodes ($p < 0.001$) and tumor size ($p = 0.006$) were also statistically significant variables in multivariate analyses. The rates of distant recurrence (DR) at 9 years in the N0 patients were 4% in the RS < 18 group, 12% in the RS 18–30 group, and 25% in the RS ≥ 31 groups and in the N+ groups, 17%, 49%, and 64% respectively. The overall survival (OS) rates at 9 years in the N0 patients were 88% in the RS < 18 group, 84% in the RS 18–30 group, and 73% in the RS ≥ 31 groups and in the N+ group, 74%, 69%, and 54%, respectively. Seventy-two N0 patients, 74 N+ patients and six node unknown patients experienced distant recurrence (DR). The DR rate increased linearly with an increase in RS. The risk was higher for N+ and for patients with ≥ 4 positive nodes. There was no significant difference in the RS and risk of DR by treatment or by N0 or N+. The prognostic information from the RS was independent of the prognostic information of Adjuvant! Online. The authors noted that the “study did not directly evaluate the value of RS in predicting the benefit of chemotherapy”, but that the data indicated that the RS adds to the information provided by node status, patient age, tumor size and tumor grade and proposed that RS may be added to treatment decisions regarding chemotherapy.” Retrospective study design, heterogeneous patient population, and the small node-positive patient population limit the ability to draw conclusions regarding the impact on health outcomes and application to patient management.

In a retrospective review of a randomized controlled trial (i.e., Southwest Oncology Group (SWOG)-8814, INT-0100 study), Albain et al. (2010) investigated the ability of the Oncotype recurrence score to determine the prognosis of node-positive (N+) women ($n = 227$) treated with tamoxifen alone and those who might not benefit from anthracycline-based chemotherapy. The study included postmenopausal women with axillary node-positive (1–3 vs. ≥ 4 nodes) breast cancer and either ER-positive or PR-positive tumors. Patients were randomized to treatment with tamoxifen alone for five years ($n = 148$; 94 with 1–3 positive nodes) or to treatment with cyclophosphamide, doxorubicin, and fluorouracil followed by tamoxifen (CAF-T) ($n = 219$; 133 with 1–3 positive nodes). Patients in this subset had a slightly lower number of positive nodes and smaller tumor size (< 2 cm [$n = 46$], 2–5 cm [$n = 94$], > 5 cm [8]) compared to the parent trial, and 11.7% were HER2 positive. The samples were analyzed using the RT-PCR Oncotype assay. The recurrence score was significantly prognostic in the tamoxifen-only group ($p = 0.006$). No benefit was identified in CAF-T patients with a recurrence score < 18 ($p = 0.97$). There was however, an improvement in disease-free survival for those with a high recurrence score ≥ 31 ($p = 0.033$). The recurrence score by treatment interaction was significant only in the first five years ($p = 0.029$), but the cumulative benefit was present at ten years. The results of the study suggested that patients with a low recurrence score and 1–3 involved axillary lymph nodes did not benefit from anthracycline-based chemotherapy, but those with a higher recurrence score had major benefit, independent of the number of positive nodes. Although the study provided further data on the prognostic value of Oncotype for post-menopausal women with ER-positive, 1–3 node-positive breast cancer treated with adjuvant tamoxifen, the authors noted the following limitations: it is unknown if the results of the study can be applied to premenopausal women; because the study involved a subset of patients from the original trial, the benefit of CAF-T at specific recurrence score values should be interpreted with caution; “the prognostic and predictive effects of the recurrence score might differ because of the inclusion of disease-free survival events such as second primary cancers and breast recurrences”.

Using tumor samples ($n = 465$) from a previous randomized controlled trial, Goldstein et al. (2008) conducted a clinical trial to evaluate the prognostic utility of Oncotype in either node-negative or node-positive, hormone receptor-positive breast cancer patients treated with doxorubicin-containing chemotherapy and to determine if Oncotype could more reliably predict outcomes at five years than standard clinicopathologic features. The study included pre- (41.4%) and postmenopausal (58.6%) women, HER2 positive (21.9%) and HER2 negative (44.0%) (HER2 status was unknown in 34.1% of women). Axillary node status included 56.5% negative nodes, 24.0% one positive node, 13.5% two positive nodes, and 6.1% three positive nodes. Tumor sizes included ≤ 2.0 cm (52.9%), 2.1–5.0 cm (42.5%), and > 5.0 cm (3.6%). All patients received chemotherapy. The median follow-up was six years. The authors used an integrator that was modeled after Adjuvant! but adjusted for five-year outcomes, evaluated the concordance between RS prediction and the integrator, compared the RS predictive accuracy with the integrator, and evaluated if RS provided additional information regarding relative risk of

recurrence. The results indicated that the RS was significantly predictive of recurrence in patients with and without positive nodes ($p < 0.001$) compared to clinicopathologic features and a clinical algorithm. Approximately 3.3% of patients with a recurrence score < 18 with 0–1 positive nodes experienced recurrence within five years compared to 7.9% with 2–3 positive nodes. Limitations of the study include the retrospective study design, use of an integrator tool developed by the authors, and the heterogeneous patient population.

Cobleigh et al. (2005) conducted a retrospective validation study which involved the analysis of paraffin block specimens from women ($n=86$) with invasive breast cancer with greater than 10 positive nodes and no evidence of metastases. Quantitative gene expression was determined by a multianalyte Taqman RT-PCR (i.e. Oncotype Dx). Seven samples were inadequate and were not used. Diagnosis included infiltrating ductal carcinoma ($n=68$) and infiltrating lobular carcinoma ($n=9$). One patient had both types of cancer. Mean tumor size was 4.4 ± 3.3 centimeters and the number of positive nodes ranged from 10–40 (median 15). A total of 54% of the women received adjuvant tamoxifen and 80% received adjuvant chemotherapy. Median follow-up was 15.1 years and median time to distant recurrent or death was 2.6 years. Only the number of nodes involved was significantly associated with distant recurrence-free survival (DRFS) ($p < 0.05$) with a 4% increase in risk for each additional involved node. HER2 expression was significantly correlated with DRFS ($p < 0.001$). The HER2/immunohistochemistry remained significant ($p < 0.05$) with regard to clinical and pathologic measurements. Clinical and pathologic variables (e.g., age, tumor size, number of involved nodes, systemic treatment) had only “modest correlation” with recurrence. The two pathologists agreed on 57.6% of the patients’ tumor grades. Analysis of the 21 genes in the recurrence score (RS) showed 14 of the 16 cancer-related genes in the RS correlated with breast cancer recurrence ($p < 0.05$ for nine genes and $p < 0.10$ for 14 genes). A total of 11 patients (14%) had a RS < 18 and a 10-year distant recurrence rate of 29%; 19 patients (24%) had a RS between 18 and 31 and a 10-year distant recurrence rate of 72%; and 48 patients (62%) had a RS of ≥ 31 and a 10-year distant recurrence rate of 80%. Overall, concordance between Oncotype DX and immunohistochemistry for ER, PR and HER2 was high, but poor for Ki-67. The authors noted that the sample size in this study for node-positive women was insufficient to “address the relative performance of the Recurrence Score and standard measures, such as patient age, tumor size, and tumor grade” and that although these data were used to develop Oncotype DX, the RS cannot be considered validated in this node-positive subpopulation.

Gianni et al. (2005) conducted a retrospective review of tissue samples from 89 patients to identify gene expression markers that predicted the likelihood of chemotherapy response. The authors also tested the correlation between chemotherapy response and the 21-gene Recurrence Score assay. Specimens were taken from women enrolled in the Istituto Nazionale Tumori (INT) of Milan (INT-Milan) trial. Fourteen patients were node-negative, 51 were one node-positive, 24 were two node-positive, 14 were supraclavicular node-positive and 62 patients were identified node-positive at surgery. After diagnostic core biopsy and before surgery, patients were treated with doxorubicin and paclitaxel. After surgery adjuvant cyclophosphamide, methotrexate, fluorouracil, locoregional irradiation, and hormonal therapy were administered. Of 384 genes tested, the expression of 86 significantly correlated with pCR ($P < 0.05$). The Recurrence Score was positively associated with the likelihood of pCR ($p = 0.005$), suggesting that the patients with a high recurrence score, are more likely to receive the greatest clinical benefit from chemotherapy.

Following a systematic review of the literature, a 2010 BlueCross BlueShield technology assessment concluded that the 21-gene OncotypeDX Breast Cancer Assay did not meet TEC criteria for gene expression profiling to aid in the selection of adjuvant chemotherapy in women with lymph-node-positive breast cancer. The assessment noted that the test is not FDA approved, and the available evidence did not allow conclusions for selecting adjuvant chemotherapy in this subpopulation. There was a lack of evidence to determine if OncotypeDX improved net health outcomes and was beneficial as an established alternative. They also stated that since it has not yet been demonstrated that the 21-gene profile improves health outcomes in the investigational setting, it cannot be demonstrated whether improvement is attainable outside the investigational setting.

Summary for Oncotype DX Breast Cancer Assay: Although published peer-reviewed evidence is in the form of retrospective analyses and observational studies, data support the accuracy and clinical utility of Oncotype in its ability to predict the benefits of chemotherapy in women with localized stage 1 or stage 2 breast cancer who are ER-positive and HER2-receptor negative with no evidence of metastasis. The patient should also be axillary-node-negative which includes micrometastasis no greater than 2.0 millimeters. Tissue samples from tumor banks were used to validate the prognostic ability of the 21-gene assay. Studies reported that patients with a low recurrence score (RS) had a 10-year distant recurrence-free survival rate of 93.2%; 85.7% for patients with an intermediate RS, and 69.5% for patients with a high RS (Tang, et al., 2011; Lo, et al., 2010; Mamounas, et al.,

2010; Toi, et al., 2010; Chang, et al., 2008; Habel, et al., 2006; Paik, et al., Aug 2006; Cobleigh, et al., 2005; Gianni, et al., 2005; Paid, et al., 2004). Supporting data on the value of repeat assays after the initial assessment are lacking. There is insufficient published peer-reviewed evidence from well-designed prospective, controlled clinical trials to support the clinical utility and predictive ability of this assay for other subsets, including women with node-positive breast cancer, and men. Furthermore, professional society/organization consensus support in the way of published guidelines or practice statements were not found for these indications.

Oncotype DX Breast Cancer Assay for DCIS: The Oncotype DX Breast Cancer Assay for DCIS (ductal carcinoma in situ) is a multi-gene diagnostic assay proposed to provide an estimate of the 10-year risk of local recurrence (DCIS or invasive carcinoma) to help guide treatment decision-making in women with ductal carcinoma in situ treated by local excision, with or without tamoxifen (GenomicHealth, 2004-2013). The DCIS Score is calculated using quantitative RT-PCR and a subset of genes obtained from the Oncotype DX Breast Cancer Assay.

Literature Review

Solin et al. (2013) reported outcomes of a validation study with the primary objective of determining “whether the 12-gene Oncotype DX DCIS Score quantifies local recurrence risk and provides risk information independent of traditional clinical and pathologic characteristics. The 21-gene Oncotype DX breast cancer assay was performed for 327 specimens(49%) from the previously published Eastern Cooperative Oncology Group (ECOG) E5194 study. Data from the 21-gene was used to calculate two separate scores for each DCIS tumor. Univariable and multivariable Cox models determined whether the DCIS Score was statistically significantly associated with IBE risk. An IBE developed in 46 patients. The 10-year IBE rates were 14.6% for cohort 1(low- or intermediate-grade DCIS, tumor size \leq 2.5 cm) and 19.0% for cohort 2 (high-grade DCIS, tumor size \leq 1 cm). The DCIS Score was significantly associated with developing an IBE when adjusted for tamoxifen use (hazard ratio [HR] = 2.31, $p = .02$). Without adjustment for tamoxifen use, the hazard ratio was essentially unchanged (HR = 2.38, $p = .01$). For invasive IBE, the hazard ratio was 3.68 ($p = .01$). Univariable analyses showed that age, menopausal status, and tumor size were statistically significant; in multivariable analyses, significant factors were DCIS Score, tumor size, and menopausal status (all $P \leq .02$). When adjusted for tumor size and menopause status the hazard ratio was unchanged which suggests that the DCIS score was an independent predictor for IBE risk. The Oncotype DX Recurrence Score(RS) was not significant for development of an IBE. Neither the DCIS nor the RS was associated with contralateral breast cancer. The retrospective study design, and cohort patient numbers limit the ability to determine the impact of the assay on patient management and overall health outcomes.

Summary for Oncotype DX Breast Cancer Assay for DCIS: Data are limited in the published, peer-reviewed literature to demonstrate the accuracy of this assay. Further there is insufficient evidence in the peer-reviewed scientific literature to support the clinical utility of the Oncotype DX Breast Cancer Assay for DCIS as a prognostic tool in an individual with DCIS.

Breast Cancer Gene Expression Ratio: The Breast Cancer Gene Expression Ratio, also known as the 2-Gene Ratio or HOXB13/IL17BR or H:I, is a breast cancer recurrence test proposed for use in “treatment-naïve individuals with ER-positive, lymph node negative breast cancer”. This RT-PCR assay is based on the ratio of expression of the homeobox gene-B13 (HOXB13) and the interleukin-17B receptor gene (IL17BR) (i.e., H:I expression ratio), and performed in formalin-fixed, paraffin-embedded tumor tissue. The results are reported as a normalized H:I expression ratio along with a categorization of low or high risk for breast cancer recurrence at 5 years. According to Quest Diagnostics, the H:I ratio serves as a continuous marker of recurrence risk in untreated patients and “should not be used to predict response to therapy. The results should be used in light of other relevant clinical and laboratory findings”. The test is licensed by Quest Diagnostics (Quest Diagnostics, 2000-2013; Marchionni, et al., 2008; Harris, et al., 2007).

The results of H:I clinical trials have indicated that “higher levels of HOXB13 and lower levels of IL17BR expression predict distant tumor recurrence in tamoxifen-treated patients with ER-positive breast cancer” and that the “H:I expression is predictive of response to first-line tamoxifen monotherapy in ER-positive breast cancer patients with metastatic disease” (Wang, et al., 2007).

Literature Review

Kok et al. (2009) conducted an analysis of 69 patients with invasive breast cancer, selected from a registry, to estimate the ability of Oncotype DX, HOXB13-IL17BR (H:I) ratio (Two Gene Index PCR), and a 78-gene microarray to predict response to tamoxifen. Patients had a relapse of disease for which first-line tamoxifen was

given for at least four weeks and had received no adjuvant systemic treatment. The number of patients classified as non-responders (i.e., patients with a time to progression [TTP] of < 6 months) or high risk was 38 using the 78-gene assay, 41 using Oncotype and four using H:I. For the 78-gene profile, the difference in median TTP for the predicted groups was 14 months (7 vs. 21) ($p=0.004$). The high-, intermediate- and low-risk groups classified by Oncotype showed a median TTP of 10, 21 and 28 months, respectively ($p=0.003$), and the two H:I groups differed by 10 months (3 vs. 13) ($p=0.004$). The high-risk Oncotype patients had worse outcomes on tamoxifen compared to the low- and intermediate-risk patients ($p=0.009$). The concordance between the three assays ranged from 45–61%. The ER, PR and HER2 status were also significantly associated with TTP. For nonresponders, the sensitivity of the 78-gene profile was 84.2%, 73.7% for Oncotype and 11.1% for H:I. However, H:I had a specificity of 95.2% compared to 46.0% for Oncotype and 56.0% for 76-gene profile. The ER sensitivity was 44.4% with a specificity of 79.6% and the PR sensitivity was 61.1% with a specificity of 63.3%. Comparison of the classifications according to the 78-gene profile to Oncotype and H:I revealed concordance with 42 and 30 patients, respectively. A total of 27 patients were classified the same by Oncotype and H:I. Assessments regarding whether the gene profiles could add predictive information to ER/PR status showed that the 78-gene profile and Oncotype had significant association independent of ER ($p=0.002$) and PR ($p=0.048$) status. Regarding the disease free interval (DFI), Oncotype ($p=0.002$) and H:I ($p=0.038$) did predict DFI, whereas the 78-gene assay did not. Based on these data, the author proposed that these gene assays could be useful prognostic indicators for TTP in this patient population and further studies are needed to investigate their clinical utility. The ability to translate these results to clinical practice is limited by the retrospective design and the small patient population studied.

Jerevall et al. (2008) conducted a study to determine if the 2-gene ratio could predict the benefit of five years vs. two years of tamoxifen treatment of 264 postmenopausal patients and investigate prognostic effects of the ratio in 93 systematically untreated premenopausal patients. Tumor samples from two different independent studies were used. The patients underwent surgery and radiotherapy if lymph node positive (LNP). A correlation was found between a high H:I ratio and larger tumors, high histological grade, and the lack of ER and progesterone receptors, and a positive HER2. IL17BR alone was correlated with factors related to poor prognosis. The lower IL17BR was associated with markers of worse outcomes. The survival curves for ER-negative postmenopausal women did not reveal any significant differences ($p=0.21$). A benefit from prolonged duration of tamoxifen was seen in postmenopausal ER-positive women with a lower H:I ratio ($p=0.021$). Likewise, a low HOXB13 with five years of tamoxifen was proven beneficial ($p=0.010$), and the benefits of longer endocrine treatment reached borderline significance ($p=0.061$). For untreated premenopausal women, a high IL17BR had better recurrence-free survival compared to a low expression ($p=0.12$), which was similar for the H:I ratio ($p=0.12$). Outcomes of the study indicated that a high H:I ratio or high HOXB13 are indicative that a patient will less likely respond to endocrine therapy and that IL17BR may be an independent prognostic factor.

Jansen et al. (2007) retrospectively measured the H:I expression levels in primary, operable tumor specimens to determine the relationship of the H:I ratio to tumor aggressiveness and response to tamoxifen. The subjects included women ($n=1252$), less than age 40 to over 79 years, premenopausal ($n=537$), postmenopausal ($n=715$), tumor sizes T1-T3, lymph node negative (LNN) and LNP, with low and high estrogen receptor (ER) and progesterone receptor (PR) status. The mRNA expression levels were measured in all 1252 specimen. The HOXB13 level showed an inverse association to IL17BR ($p<0.001$). In 448 tumors, the HOXB13 levels were significantly below detection in ER-positive tumors compared to ER-negative tumors ($p<0.001$). With the exception of tumor size, IL17BR was significantly, positively associated with age and menopausal status and negatively associated with grade and nodal status. The median expression level of HOXB13 was higher in poorly differentiated tumors, and lower in ER-positive tumors compared to ER-negative tumors. In all tumors, the H:I ratio measured as a univariate log-transformed continuous variable was associated with a poor DFS ($p<0.001$) and poor overall survival (OS) ($p<0.001$). To test for relation between expression ratio and LNN, 468 ER-positive tumors were analyzed, of whom 217 patients had a relapse during follow-up. In univariate analysis, the H:I ratio was significantly associated with a poor DFS ($p=0.001$) and a poor OS ($p<0.001$). The H:I prognostic value was assessed in 151 ER-positive lymph node positive patients and was associated with a poor DFS ($p=0.023$) and poor OS ($p<0.001$). In 193 ER-positive tumor patients treated with tamoxifen, the H:I ratio was related to a poor response ($p=0.027$), a short progression-free survival (PFS) ($p<0.001$), and poor postrelapse survival (PRS) ($p<0.001$). The study indicated that high H:I levels are associated with tumor aggressiveness and tamoxifen monotherapy failure. The authors proposed that the results of the study supported the fact that H:I expression levels were associated with tumor aggressiveness and tamoxifen failure in this group.

Using RT-PCR, Wang et al. (2007), measured HOXB13, IL17BR and CHDH gene expression and correlated it with ER and PR, known biomarkers of tamoxifen response, and HER2 expression. Formalin-fixed, paraffin-embedded tumor samples were prospectively collected from 75 consecutive ER-positive and 73-consecutive ER-negative breast cancer patients. A high HOXB13 was observed in 50% of ER-positive and 67% of ER-negative tumors ($p=0.047$). IL17BR and CHDH expressions were higher in ER-positive tumors ($p=1.8e-07$). HOXB13 correlated positively with HER2 status in ER-positive tumors ($p=0.021$) and IL17BR and CHDH negatively correlated with HER2 status, more so in ER-positive tumors ($p=0.0020$; $p=0.026$, respectively). The study indicated that HOXB13 and IL17BR are regulated by estrogen, but does not formally establish that they are direct targets of estrogen. The authors concluded that “these results provide a biological rationale for the prognostic utility of these three genes in early-stage ER-positive breast cancer and for their potential to predict anti-estrogen resistance.”

Goetz et al. (2006) studied the association of the H:I ratio to clinical outcomes of relapse and survival in ER-positive breast cancer patients enrolled in the North Central Cancer Treatment Group adjuvant tamoxifen trial (NCCTG-89-30-52), a randomized phase III trial involving women with resected ER-positive breast cancer. Postmenopausal women with node-negative disease were T1C or T2N0M0 and any age. Women who were node-positive were at least age 65 years, tumor stage T1-T2N1M0. Follow-up ranged from 5.7–13.6 years (median, 11 years). Tumor blocks were obtained from 211 patients from the tamoxifen only arm and H:I profiles were obtained from 106 patients. In LNP patients ($n=86$), the H:I ratio was not associated with relapse or survival, but in the LNN patients ($n=130$), a high ratio was associated with a worse relapse-free survival (RFS) ($p=0.031$), DFS ($p=0.015$ and OS ($p=0.014$) independent of standard prognostic markers. The study suggested that the H:I ratio was associated with relapse and survival in LNN breast cancer. The retrospective study design and small cohort population limits the ability to determine the impact of the use of this assay on patient management.

Summary for Breast Cancer Gene Expression Ratio: The evidence in the peer-reviewed literature does not support the accuracy and clinical utility of Breast Cancer Gene Expression Ratio as a predictor of tumor recurrence. The impact of this test on meaningful health outcomes has not been established. Studies are retrospective in design and include heterogeneous patient populations. The outcomes vary based on whether or not the patient is ER-positive or negative, lymph node-positive or negative, and the HER2 status. Results of clinical trials include an overall accuracy of 81%, positive predictive value of 82-87%, negative predictive values of 75–82%, and recurrence rates of low risk patients at 17–25%. Studies comparing the 2:1 Ratio to conventional risk classifiers are lacking.

HERmark[®] Breast Cancer Assay: The HERmark Breast Cancer Assay (Monogram Biosciences, South San Francisco, CA) is proposed to help determine prognosis and therapeutic choices for metastatic breast cancer. According to the manufacturer, HERmark is a dimerization assay that quantitatively measures HER2 total protein (H2T) and functional HER2 homodimers (H2D) to aid in stratifying patients with breast cancer who are likely to respond to trastuzumab (Herceptin[®])-containing therapy. Immunohistochemistry (IHC) (scored as 0, 1+, 2+, and 3+), and fluorescence in situ hybridization (FISH) are the two standard testing methods for assessing HER2 expression. “Clinical Practice Guidelines recommend determining HER2 status in patients with all invasive breast cancer, but caution that current HER2 testing methods such as central immunohistochemistry and fluorescence in situ hybridization test may be inaccurate in approximately 20% of cases (Raman, 2013).” According to Huang et al. (2010), neither IHC nor FISH “is a perfect predictor of response to trastuzumab, and both tests are affected by interlaboratory variability”. HERmark is performed using formalin-fixed, paraffin-embedded (FFPE) tissue samples. Results are reported as HER2 negative (i.e., < 5th percentile of samples classified as HER2 positive by reference methods), positive (i.e., > 95% of samples classified as HER2 negative by reference methods) or equivocal (i.e., overlap of HER2-positive and negative distributions, 95th percentile). The test is Clinical Laboratory Improvement Amendments (CLIA) validated and performed in a College of American Pathologists (CAP)-certified clinical reference laboratory (Huang, et al., 2010; Monogram Biosciences, Inc., 2013).

In a study comparing HERmark to IHC and FISH in archived samples from patients with invasive breast cancer ($n=237$), Huang et al. (2010) reported that the overall concordance between HER2 protein expression (H2T) and IHC was 67%. The positive and negative concordance between HERmark and IHC were 95% and 92%, respectively. When the equivocal cases (IHC 2+ HER2) were excluded, overall concordance was 98%. The concordance values between the negative, equivocal, and positive results from FISH compared to HERmark ranged from 39%–67%. The authors noted that “clinical studies are clearly needed to understand the relationship between quantitative HER2 expression and homodimer measurements with clinical outcomes in patients with

breast cancer treated with anti-HER2 therapy”. It was also stated that “the limited number of trastuzumab-treated cases in this study does not provide adequate statistical power for sufficient analysis of correlating the HERmark status and patient response to trastuzumab.”

Lipton et al. (2010) conducted a study to quantitatively measure HER2 expression in 102 formalin-fixed paraffin-embedded (FFPE) breast tumor specimens using HERmark and compared the results to local IHC and central FISH results, along with clinical outcomes. The specimens were from a study population of women diagnosed with metastatic breast cancer as IHC 3+ (n=95) or IHC 2+/FISH positive (n=5) or IHC unknown/FISH positive (n=2) who were prospectively observed during trastuzumab-based therapy. Follow-up ranged from 11.8-77.9 months (mean 34 months). Discordance was reported in three of 22 FISH-negative patients who were H2T expression high and 10 of 76 FISH-positive patients who were H2T expression low. The patients with H2T expression low experienced a significantly shorter time to progression on trastuzumab compared to FISH-positive patients with high total HER2 protein expression. The authors noted that “an important limitation” of this analysis was that they did not have enough material to gather central IHC data for the discordant subgroup of patients. Other author-noted limitations included the small sample size, retrospective study design, “the lack of a trastuzumab-untreated control arm, the lack of central IHC measurements for all patients to confirm that some of the cases included were not HER2 IHC false positives, and the heterogeneous chemotherapy to which patients were exposed.”

Summary for HERmark® Breast Cancer Assay: Evidence in the published peer-reviewed scientific literature has not established the accuracy, clinical utility, and beneficial impact on health outcomes of this evolving technology.

MammaPrint: MammaPrint is a 70-gene profile that classifies breast cancer into Low Risk or High Risk of recurrence, by measuring genes representative of all the pathways of cancer metastases which were selected for their predictive relationship to 10-year recurrence probability (Raman et al., 2013). From a fresh-frozen sample, the test extracts mRNA and hybridizes it to a DNA microarray. The specific genes expressed in the tumor tissue and the resulting gene expression profiles are proposed to be predictive of the risk of metastasis. MammaPrint was validated in the TRANSBIG collaborative study (n=302) (Raman et al., 2013; Agendia, 2009-2013; Buyse, et al., 2006). MammaPrint is also included in the Symphony™ Breast Cancer Profile, also offered by Agendia.

U.S. Food and Drug Administration (FDA): Approved by the FDA, MammaPrint (Agendia USA, Irvine, CA; Agendia BF, Amsterdam, The Netherlands), is a qualitative in vitro diagnostic test, performed in a central laboratory, using the gene expression profile of fresh breast cancer tissue samples to assess a patient's risk for distant metastasis (i.e., up to 10 years for patients less than 61 years old, up to 5 years for patients ≥ 61 years). Per the FDA approval, the test is indicated for breast cancer patients, with Stage I or Stage II disease, with tumor size ≤ 5.0 centimeters (cm) and who are lymph-node negative. The MammaPrint result is indicated for use by physicians as a prognostic marker only, along with other clinicopathological factors” (FDA, 2011; FDA, Feb 2007). The FDA did not require prospective clinical trials for the approval.

Literature Review

Using MammaPrint, five-year from surgery to distant metastasis (DMFS) rates have been reported at 93%-100% in low-risk groups and 80% in high-risk groups. The ten-year DMFS ranged from 87–88% in low-risk groups and 69–72% in high risk groups. The five-year and ten-year breast cancer specific survival (BCSS) in low-risk groups were 99% and 87%, respectively and 88% and 72%, respectively in high-risk groups. The negative predictive value for distant metastasis-free survival at five years in low risk groups was 100% and the negative predictive value for distant metastasis-free survival at five years in high risk groups was 94%. Reported positive predictive values have ranged from 9.8%–12% for low risk. Discordance in up to approximately one-third of the patients between the Signature and the clinical risk index used (e.g., Adjuvant! Online) has been recorded (Mook, et al., Apr 2010; Ishitobi, et al., 2010; Wittner, et al., 2008; Van De Vijver, et al., 2002). The sensitivity of the MammaPrint has been reported at 85–92% with a specificity of 42–86% (California Technology Assessment Forum, 2010).

Drukker et al. (2013) reported results of a prospective observational study (i.e., microarRAY-prognoSTics-in-breast-cancER [RASTER]) of 427 patients with a histologically confirmed unilateral, unifocal, primary operable, invasive adenocarcinoma of the breast). Patients had node negative disease. Primary objective was to compare five-year distant recurrence-free interval (DRFI) results of 70-gene signature and Adjuvant ! Online (AOL) and decisions regarding treatment. Following breast ablation or breast conserving surgery tumor samples were

frozen and used to evaluate the performance of the 70-gene signature. Treatment was based on standard Dutch guidelines, the 70-gene signature, and doctors' and patients' preferences. Fifteen percent of the 70-gene signature low-risk patients received adjuvant chemotherapy (ACT) versus 81% of the 70-gene signature high-risk patients ($p=0.03$). The five-year DRFI probabilities for the 70-gene signature low risk group ($n=219$) were 97% and 91.7% for the high-risk group ($n=208$) compared to 96.7% and 93.4%, respectively, for the AOL low- ($n=132$) and high-risk groups ($n=295$) ($p=0.24$). Ninety-three percent of 70-gene signature low-risk and AOL low-risk did not receive adjuvant chemotherapy (AST). For 70-gene signature low-risk–AOL high-risk patients ($n=124$), of whom 76% ($n=94$) had not received ACT, five-year DRFI was 98.4%. No significant difference ($p=0.29$) was seen between systemically untreated patients with a concordant low risk assessment and patients with a 70-gene signature low-risk result even with a high risk assessment by AOL. In the AOL high-risk group, 32% less patients would be eligible to receive ACT if the 70-gene signature was used. Data suggest similar DRFI probabilities between low-risk 70-gene and AOL results, however, discordant risk estimations between the 70-gene signature and AOL groups occurred in 38% of the cases. Study limitations included the uncontrolled observational design. In addition the authors note “the actual treatment decisions in this study were based on the restrictive Dutch guidelines of 2004 and doctor’s and patients’ preferences. While this reflected clinical practice at the time of the study, equality of prognosis between groups that did or did not receive chemotherapy cannot be guaranteed.”

Kok et al. (2012) retrospectively evaluated the additional value of the combined use of molecular prognostic (70-gene signature) and predictive markers (ER and PR), for outcome prediction in node-positive, ER positive, tamoxifen-treated breast cancer. Three series were evaluated: 121 patients (81% node-positive) received adjuvant tamoxifen, 151 patients did not receive tamoxifen (10%-node positive) and 92 patients received tamoxifen for metastatic disease. For the 70-gene signature the association with outcome after adjuvant tamoxifen was not significant. Data suggest that there may be some additional value for the model in which clinical parameters are combined with 70-gene signature and endocrine response categories in this study population; however, uncontrolled retrospective study design limits the ability to apply results. The authors note “Although it has been shown that the 70-genesignature is able to predict outcome in post-menopausal patients as well as in patients with node-positive disease, further research is needed in order to select patient groups in which the 70-gene signature can predict outcome after tamoxifen and consequently may be helpful in tailoring treatment decisions. Our findings are based on a relatively small dataset and confirmation by others will be crucial.” The authors also note “The 70-gene signature has not been extensively studied in patients with many positive nodes or in series of patients who all developed metastases.”

Hartmann et al. (2012) conducted a prospective observational study to evaluate the prognostic value of MammaPrint in 60 women, age ≥ 60 years, with invasive breast cancer. Clinical risk was assessed by the Adjuvant! Online (AOL) risk assessment tool. Using MammaPrint, 38 patients were considered low-risk and 22 high-risk. AOL classified 27 patients as low-risk and 33 as high-risk. Seven patients were recommended for adjuvant chemotherapy and there was discordance between AOL and MammaPrint in five of these cases. Standard adjuvant endocrine therapy for five years without chemotherapy was recommended in 53 patients and there was discordance in 24 of these cases. When MammaPrint was combined with clinico-pathologic factors, recommendation of adjuvant therapy differed in 11 patients with chemotherapy being recommended for six patients and withheld in five patients. No statistically significant differences were reported between low- and high-risk groups when MammaPrint was compared to clinico-pathological characteristics (e.g., tumor stage, node state, histological grade).

Kunz (2011) conducted a prospective case series ($n=46$) to investigate the clinical implementation of MammaPrint into the assessment of premenopausal women, age 32–56 years (mean, 44 years), with early breast cancer. The specimens were excised from the outer margin of the tumor. Tissue sampling and MammaPrint were integrated into clinical assessment of the patient. The results of MammaPrint were compared to clinical pathological factors using the St. Gallen breast cancer guidelines based on HER2 status and Adjuvant! Online. Overall, MammaPrint identified 29 low-risk patients and 15 high-risk patients compared to four low risk, 34 intermediate risk and six high risk by St. Gallen method and 19 low risk and 25 high risk by Adjuvant! Online. MammaPrint identified 13 low risk and five high risk patients with node-positive disease compared to 17 low risk and nine high risk patients with node-negative disease. The author noted no issues incorporating MammaPrint into the evaluation of this patient population. No information about clinical utility and outcomes based on MammaPrint were reported. Limitations of the study include the small patient population, lack of follow-up and lack of information regarding clinical utility of MammaPrint.

In the same publication above, Kunz (2011) also conducted a pooled analysis of 650 patients, age range 35–55 years, from three retrospective studies to assess the accuracy of MammaPrint. A total of 2.5% of the patients in the node-negative group (n=169) received adjuvant chemotherapy, compared to 83% in the node-positive group (n=511) who received hormone therapy alone (16%), chemotherapy alone (40%) or both therapies (27%). MammaPrint stratified 248 patients as low risk and 402 patients as high risk. MammaPrint predicted the 10-year overall survival probability for the low risk patients as 90.2% and 65.2% for high risk patients. A significant difference was reported for the probability of remaining metastasis free and for overall survival with MammaPrint (p<0.001). St. Gallen guidelines characterized 522 of 650 patients at intermediate risk with a 77.2% 10-year overall survival and a 76.4% distant metastases free survival. According to MammaPrint, the intermediate group was stratified as 58% high risk with a 10-year survival probability of 60.9% and 42% low risk with a ten-year survival probability of 91.4%.

Gevensleben et al. (2010) conducted a validation study of MammaPrint and TargetPrint (Agendia, Irvine, CA) using data bank, frozen breast tissue from women (n=140) with stage I and stage II breast cancer. A total of 134 patients received adjuvant chemotherapy, endocrine therapy or both. Using MammaPrint the patients were classified as low risk (poor prognosis) (n=62) or high risk (good prognosis) (n=78) for distant metastasis and the results were compared to clinical risk classifications and adjuvant treatment management. The TargetPrint results were compared to immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), and chromogenic in situ hybridization (CISH). Of the 123 patients classified as intermediate risk by St. Gallen, 53 were classified as poor prognosis by MammaPrint and 70 as good prognosis. Two high-risk patients per St. Gallen had a good prognosis per MammaPrint. Compared to Adjuvant! Online, there was discordance in 57 patients with 45 Adjuvant! high-risk patients classified as good prognosis with MammaPrint and 12 Adjuvant! low-risk patients classified as MammaPrint poor prognosis. Patients with a poor prognosis signature were more prone to have a larger size tumor with a high histological grade and be ER/PR negative. Nineteen patients with a MammaPrint poor prognosis-signature did not receive adjuvant systemic treatment except for endocrine therapy and according to the authors were potentially undertreated. Thirty-five patients who received chemotherapy were categorized as good prognosis with MammaPrint and were potentially overtreated. The TargetPrint analyses were concordant with IHC/FISH/CISH 97% of the time for ER; 86% for PR; and 94% for HER2. TargetPrint classified two samples as negative that were IHC 2+ for HER2 and not scored by FISH.

Knauer et al. (2010) conducted a retrospective review of 89 patients selected from a database to determine if MammaPrint could identify HER2 positive patients with favorable outcomes. Included patients met the following criteria: unilateral stage pathological (p) T1–3, lymph node negative or one positive node, metastasis-free, HER2 positive invasive breast carcinoma, no neoadjuvant chemotherapy, and surgical treatment, radiotherapy and/or adjuvant systemic therapy (i.e., 36 patients received adjuvant endocrine therapy) as indicated. Follow-up ranged from 4–303 months (median, 65 months). Distant recurrence occurred in 49 patients and 41 experienced breast cancer-specific deaths. MammaPrint classified 20 patients as good prognosis with 10-year distant disease-free survival (DDFS) of 84% compared to 69 poor prognosis patients with DDFS of 55%. The HER2 positive patients characterized as MammaPrint good prognosis were more likely to have lobular cancers (p=0.002) and less likely to be histological grade 3 (p<0.001). No significant differences were found regarding “age, tumor stage, lymph node involvement, ER and PR status and type of surgery.” Compared to the poor prognosis patients, a significant fewer number of patients in the good prognosis group received adjuvant endocrine therapy (p=0.041). Adjusted for known prognostic factors and endocrine treatment, MammaPrint and tumor size were independently correlated with 10-year distant metastasis-free and breast cancer-specific survival (BCSS). “To explore whether expression of hormonal receptors identified a subgroup of HER2 positive/70-gene profile low-risk tumors with a particularly favorable outcome” the authors analyzed a subgroup of 40 patients with HER2 overexpression that were highly responsive to endocrine therapy, of which 21 did not receive chemotherapy. No distant metastases or breast cancer-specific deaths were observed in MammaPrint good prognosis patients (n=11). The authors noted that limitations of the study included the small patient population, short-term follow-up, heterogeneous population with or without endocrine therapy, as well as the retrospective analysis, which could not account for all potential biases in treatment selection. The author’s noted that further validation of MammaPrint’s ability to identify low-risk HER2 positive patients who could receive less intensive therapy are ongoing in the Microarray In Node negative Disease may Avoid ChemoTherapy (MINDACT) trial.

Straver et al. (2010) conducted a retrospective analysis of fresh frozen tumor biopsies and clinical data (e.g., tumor size and grade, node status, ER/PR and HER2 status) on 167 consecutive patients treated with neoadjuvant chemotherapy for invasive breast cancer > 3 centimeters, stage II-III and/or involved lymph nodes. The biopsies were taken prior to chemotherapy. Overall, following completion of chemotherapy, appropriate

surgical intervention occurred. MammaPrint was used to assess the mRNA expression level of the 70 genes and tumors were classified as a good signature prognosis (low risk) or poor signature prognosis (high risk). The primary outcome measure was the “absence of invasive carcinoma in both the breast and axilla at microscopic examination of the resection specimen, regardless of the presence of carcinoma in situ” (i.e., pathological complete response [pCR]). The response of the primary tumor was also reviewed and was considered pCR when no residual tumor cells were seen microscopically or as near pCR (npCR) when a small number of scatter tumor cells were present or tumor cells in an area of < 2 millimeters in diameter were present. Follow-up ranged from 5–91 months (median 25 months). Twenty-three patients had a good signature prognosis and 144 patients had a poor signature prognosis. Poor prognosis typically involved higher grade tumors classified as triple-negative (i.e., ER-, PR- HER2-negative) or HER2 positive. All 38 triple negative tumor patients had a poor prognosis signature. The response of the primary tumor in the nontriple-negative tumor patients remained associated with the classification of the prognosis signature ($p=0.023$). Many of the poor prognosis patients were treated with a trastuzumab (Herceptin™) based regimen. The overall pCR rate was 17% (29/167), none in the good prognosis-signature patients ($n=23$) and 20% of the poor prognosis patients (29/144; $p=0.015$). Chemosensitivity was assessed by separately analyzing the pathological response (i.e., pCR, npCR) of the primary tumor. Two good prognosis patients achieved near pCR of the primary tumor while 53 poor prognosis patients were significantly associated with the pCR ($p=0.008$). Patients with a low MammaPrint Index had a higher probability to achieve pCR and were more sensitive to chemotherapy.

Mook et al. (May 2010) evaluated the accuracy of the 70-gene MammaPrint signature on 964 frozen samples from seven previously reported studies on women with pT1 tumors (≤ 2 cm). A total of 139 patients had a pT1ab tumor (≤ 10 mm), 825 had a pT1c (11–20 mm) tumor, 693 patients had node-negative cancer, and 263 patients had node-positive cancer. Of the 964 patients, 552 received no adjuvant systemic therapy, 408 patients received endocrine and/or chemotherapy, and adjuvant systemic therapy was unknown for four patients. Follow-up ranged from 0.2–25.2 years (median 7.2 years), and during this period 154 patients developed distant metastases and 130 patients died of breast cancer. Twenty-five patients died of other causes. Outcomes included time from surgery to distant metastasis (DMFS), and time from surgery to breast cancer related death (i.e., breast cancer specific survival [BCSS]). MammaPrint classified 525 tumors as good prognosis and 439 as poor prognosis. For the good prognosis group, the 10-year DMFS rate was 87% and the BCSS was 87%. The probability of remaining free of distant metastases at 5 years was 95% and at 10 years was 87%. The five- and ten-year BCSS was 99% and 91%, respectively ($p<0.001$). The DMFS and BCSS for the poor prognosis group was 72% each at ten years. MammaPrint was an independent prognostic factor for BCSS at 10 years ($p<0.001$) and additionally, predicted DMFS at 10 years ($p=0.04$) for 139 patients with pT1ab cancers. The probability of remaining free of distant metastases at 5 years was 80% and at 10 years was 72%. The five- and ten-year BCSS was 88% and 72%, respectively ($p<0.001$). Of the patients with pT1ab tumors, 40% were classified as poor prognosis. The signature retained its prognostic value in untreated patients and was an independent prognostic factor in 788 ER-positive patients for DMFS and BCSS ($p<0.001$, each). It was noted that a potential limitation of the study was the heterogeneous patient population, especially related to years of diagnosis and adjuvant therapy and some patients had been previously treated based on the outcome of MammaPrint. Other limitations included the small patient populations in the T1ab subgroups, and retrospective study design.

In the first study to assess the prediction of adjuvant chemotherapy using MammaPrint, Knauer et al. (2010) evaluated the predictive value of this test for 315 patients treated with endocrine therapy (ET) compared to 226 patients treated with endocrine plus chemotherapy (CT). Specimens were selected from a database from seven previously reported studies and included patients with unilateral stage pT1-3, node 0–1 breast cancer with no distant metastasis. Patients were ER-positive (90%) and PR-positive (69%). Follow-up ranged from 0.2–25.2 years (median 7.2 years). At five years, 52 patients had distant metastases and 33 had died of the disease. MammaPrint classified 252 patients as low risk and 289 patients as high risk. At five years, the BCSS for the low-risk group was 97% for the ET group and 99% for the ET/CT group ($p=0.62$) and the DDFS was 93% and 99%, respectively. The BCSS for the high-risk group was 81% for the ET group and 94% for the ET/CT group ($p<0.01$). The DDFS was 76% and 88%, respectively. Significant survival benefit was shown with the addition of CT in the high-risk group. Patients in the low-risk group gained no significant benefit from the addition of chemotherapy. Limitations of the study include the retrospective design, small patient populations, and difference in CT regimens.

Using frozen tumor samples ($n=241$), Mook et al. (2009) conducted a retrospective validation study to investigate the ability of MammaPrint to accurately identify patients with unilateral T1, T2 or operable T3 invasive breast cancer with 1–3 positive lymph nodes (including micrometastasis [$n=29$ micrometastasis only]) who had an

excellent disease outcome. Eligible patients had no prior malignancies, no bilateral synchronous breast tumors and received no adjuvant therapy. Treatment included surgical incision and dissection of axillary lymph nodes, radiotherapy and when indicated, adjuvant systemic therapy (i.e., endocrine therapy, chemotherapy, both). Follow-up ranged from 0.01–12.3 years (median 7.8 years). Using MammaPrint, 99 patients (41%) were classified as good prognosis signature and 142 (59%) were poor prognosis signature. Poor prognosis patients more often received adjuvant chemotherapy and less often endocrine therapy. Poor prognosis tumors were larger, poorly differentiated, ER- and PR-negative and HER2/NEU positive. Sixty-six patients had at least one event (e.g., recurrence, contralateral breast cancer, secondary primary cancer, metastases, death). Breast cancer specific survival (BCSS) and distant metastasis as first event (DMFS) were significantly better in the good prognosis group. The five- and ten-year BCSS probabilities were 99% and 96%, respectively for the good prognosis signature patients compared to 88% and 76%, respectively for the poor prognosis signature patients. Shorter BCSS was seen with a poor prognosis ($p=0.001$). The probability of remaining free of distant metastases at five- and ten-years were 98% and 91%, respectively for good prognosis compared to 80% and 76%, respectively for poor prognosis ($p=0.02$). MammaPrint ($p=0.001$), as well as the number of positive nodes, tumor grade, ER status, HER2/Neu status were significantly predictive of BCSS. One analysis showed that MammaPrint was the most independent predictor of BCSS ($p=0.005$). Adjuvant! Online classified 32 patients as clinical low risk and 209 patients as high risk. This clinical risk assessment was discordant with MammaPrint by 77 patients (i.e., five classified as clinical low risk were poor prognosis signature and 72 clinical high risk were good prognosis signature). In the 27 patients identified as good prognosis signature and clinical low risk, none developed distant metastases or died. When the clinical high risk group ($n=209$) was stratified by signature risk, the 10-year BCSS probability was 94% for the good prognosis-signature group and 76% for the poor prognosis-signature group. MammaPrint was also predictive for BCSS in the 101 chemotherapy naïve patients ($p=0.001$), the 128 chemotherapy treated patients ($p=0.04$), 63 endocrine naïve patients ($p=0.001$), 166 endocrine treated patients ($p=0.02$), and 191 ER-positive tumors ($p=0.002$). In the 106 additional samples from another study with 1–3 node positive node patients, the 10-year BCSS probability was 98% for good prognosis and 64% for poor prognosis and a poor prognosis was associated with shorter BCSS ($p=0.002$). When the data from additional 106 were added to a pooled analysis of the data from this study, the hazard ratio for DMFS, as first event, remained consistent for MammaPrint ($p=0.009$). Study limitations include the uncontrolled retrospective design.

Bueno-de-Mesquita et al. (2007) conducted a prospective study to assess the feasibility of implementation of the MammaPrint assay in a community-based setting and to determine its effect on adjuvant systemic treatment decisions compared to treatment advice provided by the Dutch Institute for Healthcare Improvement (CBO) and other guidelines. The Microarray Prognostics in Breast Cancer (RASTER) study included 427 viable samples that met inclusion criteria from women, under age 61 years, with primary, unilateral, operable, invasive adenocarcinoma of the breast. Follow-up ranged from 0.3-36.4 months (median 14 months). The study protocol was amended at the end of 2004 to include women less than age 55 years. MammaPrint (i.e., signature) identified 219 patient with good prognosis and 208 patients with poor prognosis compared to 184 poor prognosis identified by the CBO guidelines which was discordant with 128 signature results. Adjuvant therapy would be initiated in 203 patients based on CBO guidelines; 265 patients based on MammaPrint results; and 259 patients based upon CBO, MammaPrint and patient preferences. Adjuvant! Online identified 294 patients with poor prognosis (discordant with 160 signature patients) and St. Gallen guidelines with the signature identified 353 patients with poor prognosis. The Nottingham Prognostic Index with the signature identified 179 poor-prognosis patients, discordant with 117 patients. Discordance was noted in approximately one-third of the patients between the signature and the clinical risk index regardless of the index used.

The California Technology Assessment Forum (CATF) (2010) conducted a systematic review of the literature to evaluate the evidence of MammaPrint. Earlier studies reported a sensitivity of 85%-92% and a specificity of 73%-86%. The Forum noted that the strength of the association for DMFS was strongest in studies with short-term follow-up and weakest in studies with long-term follow-up, suggesting that MammaPrint is primarily a risk factor for metastasis during the first-five years of follow-up. The authors concluded that there was insufficient evidence to demonstrate that MammaPrint improves net health outcomes and is as beneficial as established alternatives. Improvement outside of the investigational setting has not been reported. Therefore the CTAF does not recommend MammaPrint.

Summary for MammaPrint: The evidence in the published peer-reviewed scientific literature does not support the accuracy and clinical utility of MammaPrint for the prognosis of breast cancer. Studies have included retrospective analysis of tissue samples from case series with heterogeneous patient populations and variable follow-up lengths. It is yet to be proven in prospective, clinical trials that MammaPrint improves stratification

beyond what is currently available through clinical and histopathological assessment or that it provides meaningful improvements in health outcomes.

Other Gene Expression Assays

A number of additional gene assays, including the Rotterdam Signature 76-Gene Panel, Blue Print™ Molecular Subtyping Profile (Agendia, Irvine, CA), Breast Cancer Gene Expression Prognosis Profile (BreastOncPx™, (Integrated Oncology, Laboratory Corporation of America, Irvine, CA), Breast Cancer IndexSM: Breast Cancer Index , (bioTheranostics, San Diego, CA), Clariant InsightDx® Mammostrat (Clariant, Inc., Aliso Viejo, CA), and the PAM50 formerly Breast Bioclassifier™ (ARUP Laboratories, Salt Lake City, UT), have been proposed to aid in treatment planning and predicting prognosis of women with various types of breast cancer. Some assays are awaiting FDA approval, while others are still in the developmental stages and are not commercially available. High-level controlled prospective trial data are limited in the published peer-reviewed literature and accuracy and clinical utility of these assays has not been established. The role of these assays in the management of individuals with breast cancer and their impact on health outcomes is unknown at present.

Rotterdam Signature 76-Gene Panel: The Rotterdam Signature 76-gene panel was developed to assist physicians to predict the likelihood that a patient with early-stage breast cancer will develop a metastasis. The microarray assay represents a prognostic molecular marker that is proposed to be used with all lymph node negative (LNN) breast cancer patients, regardless of age, tumor size and grade, or ER status. Sixty genes evaluate ER-positive samples and 16 genes evaluate ER negative samples. The 76-gene signature analyzes fresh frozen tumor samples and classifies patients as having a gene expression signature associated with either a low or high risk of developing metastatic disease. The test is not yet commercially available.

Literature Review: The evidence in the published peer-reviewed scientific literature does not support the accuracy and clinical utility of the Rotterdam Signature test, nor have the data shown an impact on meaningful health outcomes in predicting the risk of breast cancer recurrence. The reported sensitivity of the test ranged from 80%-93% and the specificity ranged from 40-48%. Reported five-year distant metastasis-free survival rates included 90%–98% for low-risk patients and 74%–76% for high-risk patients. The 10-year rates were 94% for low-risk patients and 65%–72% for high-risk patients. The positive predictive value was 38%, and the negative predictive value was 94% (Desmedt, et al., 2007; Foekens, et al., 2006; Wang, et al., 2005).

Using the 76-gene signature, Zhang et al. (2009) assessed the benefit of adjuvant systemic tamoxifen therapy (n=136) vs. no therapy (n=164). Frozen tumor specimens from women with lymph node negative, estrogen positive breast cancer from two matched cohorts were analyzed. Follow-up time of surviving patients ranged from 29–193 months (median 90 months). When applied to the 136 tamoxifen-treated patients, the gene signature stratified the patients into high-risk and low-risk groups. The 10-year distant metastasis-free survival (DMFS) rate of the low-risk group was 96% compared to 77% for the high-risk group. The low-risk tamoxifen-treated patients had a non-significant (7.2%) better 10-year DMFS than the untreated patients. When the gene signature was applied to all patients, the low-risk group (n=116) showed a 2.7% non-significant 10-year DMFS benefit from tamoxifen therapy. The high-risk group (n=184) showed a significant (p=0.0318) 10-year DMFS absolute benefit of 12.3% with tamoxifen. Also, in the high-risk group, the 10-year DMFS for untreated patients was 64.7% compared to 77.0% for tamoxifen-treated patients. The authors noted that “caution should be taken to decisively interpret these results as an effect of tamoxifen therapy because the included patients did not participate in a randomized trial, but received local treatment according to institutional guidelines effective at the time of surgery”.

Desmedt et al. (2007) reported the results of a study conducted by TRANSBIG (i.e., network for improved treatment tailoring established by the Breast International Group [BIG] [TRANSBIG]), a transnational research network involving 40 partners in 21 European countries (TRANSBIG, 2006) to validate the 76-gene prognosis signature and to compare the signature outcome with clinical risk assessment. Frozen tissue samples (n=198) from a previous study (Buyse, et al., 2006) were obtained from women, age less than 61 years, with node negative, T1-T2 (≤ 5 cm) tumors. Median follow-up was 14 years, distant metastases occurred in 51 patients, and 35 patients demonstrated progression within five years. Based on the signature, patients were identified as high (n=143) or low (n=55) genomic risks and as high (n=152) and low (46) clinical risks based upon Adjuvant! Online. The low genomic risk included 14 ER-negative patients, whereas there were none in the low-risk clinical group. Fourteen low-risk genetic patients were ER-negative compared to 50 high-risk genetic patients. The gene signature actual five- and ten-year time to distant metastases (TDM) was 98% and 94%, respectively, for the good profile group and 76% and 73%, respectively for the poor profile group. The overall survival for the good

profile group was 98% and 87% at five and ten years, respectively and 84% and 72%, respectively for the poor profile group. The five- and ten-year sensitivity for TDM was 97% and 93%, respectively, with a 34% and 31%, respectively for specificity. The HR was 5.78 (95% confidence interval [CI], 1.78–18.80) for TDM and 2.87 (95% confidence interval [CI], 1.21–6.82) for OS. Adjusted for clinical risk, the global HR was 5.11 (1.57–16.67) for TDM and 2.55 (1.07–6.10) of OS). In the low genomic and low clinical risk groups, no patient developed distant metastasis.

Blueprint™ Molecular Subtyping Profile: Blueprint (Agendia, Irvine, CA) is an 80-gene assay proposed to classify breast cancer into basal-type, luminal-type or ERBB2-type (HER2/neu positive) cancers. These cancers may have various prognosis and treatment responses to endocrine or chemotherapy based on their molecular subtype. Blueprint is proposed to be used in conjunction with MammaPrint to predict which patients will benefit from endocrine therapy and which will benefit from chemotherapy (Agendia, 2004-2013; Krijgsman, et al., 2011).

Literature Review: Krijgsman et al. (2011) developed an 80-Gene Molecular Subtyping Profile (Blueprint) using 200 specimens selected for their concordance based on ER, PR and HER2 status. Results were compared to ER, PR and HER2 status identified by immunohistochemistry (IHC) and chromogenic in situ hybridization (CISH). In the validation cohorts (n=95–100), Blueprint was concordant with IHC (+ CISH for HER2) as follows: ER-positive 68%–84%; PR-positive, 46%–49%; and HER2-positive 13%–38%. The 80-gene test was confirmed using four independent validation cohorts (n=784) based on molecular subtypes (i.e., basal type, luminal type and HER2 type). Blueprint was also tested as a chemotherapy-response predictor on 133 patients treated with T/FAC (paclitaxel and fluorouracil + doxorubicin + cyclophosphamide) neoadjuvant chemotherapy. The molecular subtypes were combined with the MammaPrint based prognosis and the following four groups were identified: MammaPrint low-risk/luminal-type and MammaPrint high-risk luminal-type, basal-type, and HER2-positive. Out of all of the MammaPrint low-risk samples 92% were luminal and the high-risk were distributed in 46% luminal-type, 26% basal-type, and 28% HER2-type. These classifications were also confirmed in a separate cohort with chemotherapy responsive data and 86% of the low-risk samples were luminal compared to 53% luminal high risk.

Breast Cancer Gene Expression Prognosis Profile (BreastOncPx™): BreastOncPX (Integrated Oncology, Laboratory Corporation of America, Irvine, CA) is a 14-gene signature assay proposed for use in lymph node negative, ER-positive patients to estimate the likelihood of recurrence including distant metastasis (2012). According to the manufacturer's website, this assay was validated in untreated and tamoxifen-treated women. Tutt et al. (2008) reported that the sensitivity and specificity of the metastasis score (MS) high and low risk groups to predict distant metastases were 96% and 43% at five years, respectively, and 93% and 46% at ten years, respectively. Sensitivity and specificity of the MS risk groups to predict death from any cause at 10 years were 84% and 45%, respectively.

Breast Cancer IndexSM: Breast Cancer Index ([BCI], bioTheranostics, San Diego, CA), a combination of the Theros H/ISM (HOXB13:IL17BR, formerly AviaaraSM H/I) and the bioTheros MGISM (Molecular Grade Index, formerly Aviaara MGISM), assesses tumor grade. It is marketed as a prognostic biomarker that provides quantitative assessment of the likelihood of distant recurrence in patients diagnosed with estrogen receptor-positive, lymph node-negative breast cancer (Raman, 2013). BCI is not FDA approved. Results using the combined testing are reported as low-, intermediate- or high-risk for recurrence. The H/I and the MGI tests can be used independently (bioTheranostics, 2013).

Literature Review: Jankowitz et al. (2011) conducted a retrospective analysis of tumor samples from 265 ER+, lymph node negative, tamoxifen-treated patients to validate BCI and compared its prognostic utility to the Adjuvant! Online (AO) actuarial tool. BCI categorized 54.7% of patients as low risk, 21.1% as intermediate risk and 24.2% as high risk. Overall, the BCI 10-year rates of distant metastasis-free survival were 93.4% for low-risk, 87.9% for intermediate-risk, and 68.1% for high-risk. The overall survival rates were 93.3% for low-risk, 92.0% for intermediate risk and 71.8% for high risk. When BCI was included in the model of known prognostic factors, BCI was highly significant and was associated with recurrence risk (p=0.0002), all-cause mortality (p<0.0001), and breast cancer-specific mortality (p<0.0001). Both AO and BCI were significantly associated with risk of recurrence, all-cause mortality and breast cancer-specific mortality. Analysis revealed that for early time points (< 4 years), the model scores provided limited differential ability between AO compared to AO+BCI. For time periods greater than four years after diagnosis the predictive accuracy for recurrence increased with AO+BCI compared to AO only. The addition of BCI to AO increased predictive accuracy in all patients from 66% (AO only) to 76% (AO+BCI) for 10-year outcomes and in tamoxifen-only treated patients from 65% to 81% in the 4–10

years following diagnosis. Author-noted limitations of the study include the retrospective, single-center design and the results may have been biased based on the basis of specimen availability and patterns of referral to the institution.

Mathieu et al. (2011) conducted a retrospective review on 150 tumor samples from breast cancer patients, treated with neoadjuvant chemotherapy, to assess the ability of Breast Cancer Index (BCI) to predict chemosensitivity based on pathological complete response (pCR) and breast conservation surgery (BCS). Patients had an infiltrating breast carcinoma treated with neoadjuvant anthracycline and/or taxane. BCI classified 42% of patients as low risk, 35% as intermediate risk and 23% as high risk. The low BCI risk group had a 98.4% negative predictive value (NPV) for pCR compared to 86% NPV for BCS. The high versus low BCI group had a 34 and 5.8 greater likelihood of achieving pCR and BCS, respectively ($p=0.0055$; $p=0.0022$). BCI significantly increased the concordance index for pCR ($p=0.017$) and BCS prediction ($p=0.027$) beyond clinicopathologic factors (i.e., estrogen receptor [ER], progesterone receptor [PR], HER2, and tumor size and grade). High NPVs indicate that BCI could be a useful tool to identify breast cancer patients who are not eligible for neoadjuvant chemotherapy. These results suggested that BCI could be used to assess both chemosensitivity and eligibility for BCS. The results of this study need to be validated in prospective randomized controlled trials with large patient populations.

To demonstrate the prognostic utility of the Breast Cancer Index (BCI), Jerevall et al. (2011) conducted a retrospective analysis of tumor samples from ER-positive women ($n=588$) with invasive breast cancer who were treated with tamoxifen for 2–5 years ($n=314$) or not treated ($n=274$). Tumor specimens were taken from two earlier studies. Analysis of the tumor showed that H: I plus molecular grade index (MGI), using a low, intermediate and high risk stratification algorithm, was significantly associated with distant recurrence and breast cancer death. Over 50% of the tamoxifen-treated women were categorized as low risk and had < 3% 10-year distant recurrence or death risk. The additional tamoxifen-treated women were identified as 23% intermediate risk and 18% high risk. The H:I plus MGI index also demonstrated prognostic utility for the untreated women ($p=0.0004$) of which 50% were low risk, 27% intermediate risk and 23% high risk. The rates of breast cancer-specific death for the low-, intermediate- and high-risk groups in the untreated women were 5.3%, 19.3% and 26.3%. Based on the data for the ER-positive tamoxifen-treated women, the authors developed a continuous algorithm to compute a continuous risk index (i.e., Breast Cancer Index). Low risk was defined as $BCI < 5$; $BCI \geq 5$ but < 6.4 was intermediate risk; and $BCI \geq 6.4$ was high risk. BCI classified 59.6% of the tamoxifen-treated women as having low risk of recurrence, 22.0% as intermediate risk and 18.4% as high risk. Statistical estimates of distant recurrence were 17.8% for intermediate risk and 20.0% for high risk and 10-year rate of breast cancer-specific death were 14.5% and 14.7%, respectively. BCI was then tested by analyzing specimens from the women in the untreated arm. A total of 53% were classified as low risk, 27% as intermediate risk and 20% as high risk. The rate of distant metastasis at 10 years in these risk groups was 8.3%, 22.9% and 28.5%, respectively. The rate of breast cancer-specific death was 5.1%, 19.8%, and 28.8%. Based on these results, BCI was proposed to be a “strong prognostic factor for distant recurrence independent of tumor size, grade, PR status and HER2 status.” Tumor size did contribute to the prognostic value. BCI was also predictive of breast cancer specific death applying a similar multivariate model. Risk assessment in the tamoxifen-treated women based on the St. Gallen’s guidelines categorized 22% as low risk with a 5.2% recurrence rate and 78% as intermediate risk with an 8.5% recurrence rate. In the untreated women, 19% were low risk with a recurrence rate of 8.8% and 81% were intermediate risk with a recurrence rate of 17.0%. Compared to Adjuvant! Online, both BCI and Adjuvant! Online were significant predictors of distant recurrence and death. There was a correlation between BCI and tumor size and grade and HER2 status. Significantly more women in the low-risk group compared to the high risk group had low grade tumors ≤ 2 millimeter and HER2 negative.

Mammostrat®

Clariant InsightDx® Mammostrat (Clariant, Inc., Aliso Viejo, CA) estimates the risk for recurrence in hormone-receptor positive, early stage breast cancer that is independent of proliferation and grade. Mammostrat uses immunohistochemistry stains to stratify estrogen receptors using five antibodies. According to Clariant, the test “measures the presence of five proteins that are thought to be associated with cell cycle regulation (p53 and HTF9C), differentiation (CEACAM5), hypoxia (NDRG1), and nutrient supply (SLC7A5)”. The test is proposed for postmenopausal, node negative, ER-positive women who will receive hormonal therapy and may be candidates for adjuvant chemotherapy. The test identifies low-, moderate- and high-risk groups. Mammostrat is currently available from a centralized CLIA laboratory (Clariant, 2013; Ross, et al., 2008; Ring, et al., 2006).

Literature Review: Bartlett et al. (2010) conducted a validation study to evaluate the efficacy of the Mammostrat assay using a cohort of patients (n=1540) with breast cancer and reported that increased Mammostrat scores were associated with reduced distant recurrence-free survival (DRFS); relapse-free survival (RFS), and overall breast cancer specific survival (OS). Increased Mammostrat scores were found to be significantly ($p < 0.00001$) associated with reduced DRFS, RFS, and OS in ER-positive breast cancer patients. The risk score was independent of conventional risk factors for DRFS, RFS and OS. The 10-year recurrence rates in tamoxifen-treated, node-negative patients were $7.6 \pm 1.5\%$ in the low-risk group and $20.0 \pm 4.4\%$ in the high-risk group.

PAM50 Breast Cancer Intrinsic Classifier™

PAM50 (ARUP Laboratories, Salt Lake City, UT), formerly Breast Bioclassifier™) is a qRT-PCR assay that measures the expression of 50 classifier genes and five control genes to identify four breast cancer tumor subtypes (i.e., luminal A, luminal B, HER2-enriched, basal-like). The test is proposed for patients with invasive breast cancer, regardless of the stage or ER status to aid in determining treatment.

Literature Review: Chia et al. (2012) conducted a retrospective review of tumor samples from a randomized controlled trial to evaluate the prognostic and predictive value to adjuvant hormonal therapy of PAM50 compared to immunohistochemistry (IHC) in pre-menopausal women with primary breast cancer. A total of 398/672 (59%) RNA samples were available for PAM50 intrinsic subtyping for luminal A, luminal B, HER-2 enriched and basal like subtypes. A tissue microarray was constructed from 492/672 (73%) to assess a panel of six IHC antibodies (i.e., estrogen receptor [ER], progesterone receptor [PR], HER-2, antigen Ki67, cytokeratine [CK] 5/6 and epidermal growth factor receptor [EGFR]) to identify the same intrinsic subtypes. The concordance for intrinsic subtypes among 348 patients who could be classified by both IHC and PAM50 classifiers was 82.2%, 81.0%, 87.6%, 97.4% for luminal A; luminal B; HER-2 enriched (defined by PAM50)/luminal B HER2+ and ER-/PR-/HER2+ (defined by IHC); and basal-like breast cancers, respectively. Classification into intrinsic subtypes by the PAM50 assay was prognostic for disease free survival (DFS) ($p = 0.0003$) and overall survival (OS) ($p = 0.0002$) but not by IHC. Luminal subtyping by PAM50 was predictive of tamoxifen benefit for non-luminal subtypes, but was not statistically significant ($p = 0.24$). Neither subtyping by central IHC nor by local ER or PR status was predictive.

Kelly et al. (2012) retrospectively analyzed tumor samples in 108 women with early stage, ER+ breast cancer to compare risk assignment by PAM50 to risk assignment by Oncotype. Analysis of the distribution of the Oncotype recurrence score (RS) values and risk groups across PAM50 intrinsic subtypes showed that luminal A cancers had a significantly lower median RS value (RS=15) than luminal B (RS=25) ($p < 0.001$) cancers or any other subtype. The highest RS value (57) was observed in single basal-like cancer. The distribution of histologic tumor grade across RS and PAM50 risk groups showed that luminal A cancers and the low and intermediate risk RS groups included approximately equal numbers of grade I and grade II cancers. When grade II tumors (n=61) were classified by Oncotype and PAM50, 56% of tumors were classified as low RS risk and 63% as luminal A. The distribution of PAM50 intrinsic subtypes across the RS groups included: ten high risk patients with nine luminal B and one basal-like subtypes; 39 intermediate risk with 23 luminal A and 13 luminal B and three HER-2 enriched; and 59 low risk RS with 53 luminal A, five luminal B and one HER2 enriched. There was good agreement between the two assays for high RS and luminal B and low RS and luminal B. However, PAM50 assigned more patients to the low risk category and assigned about half of the intermediate risk RS group to the luminal A low risk.

From frozen tumor samples (n=786), Nielsen et al. (2010) conducted a validation study and compared the results of clinicopathologic indicators, immunohistochemical (IHC) and PAM50 to determine the best approach for analyzing the prognosis of women with ER-positive breast cancer, metastasis-free, with various stages of cancer who had zero to ≥ 10 positive lymph nodes. Patients received local treatment, five years of tamoxifen (no adjuvant chemotherapy) and were followed for relapse-free survival (RFS) and disease-specific survival (DDS) for over ten years (median follow-up 11.7 years). PAM50 analysis identified the following subtypes: 372 luminal A, 329 luminal B, 64 HER2-enriched, 5 basal-like and 16 normal-like. In this cohort all samples were ER-positive per IHC and 98.8% positive for dextran-charcoal-coated (DCC) biochemical assay. However, these results were discordant with the nonluminal subtypes who were mostly HER2-enriched. Patients who received tamoxifen had tumors mostly node positive, high grade with lymphovascular invasion and constituted a higher-risk group with an overall 10-year RFS of 62% and DSS of 72%. Those assigned by the PAM50 assay to luminal A status had a significantly better outcome with a 10-year RFS of 74% and a DSS of 83% compared to the luminal B, HER2-enriched, or basal-like tumors. Despite clinical ER positivity, 10% of cases were assigned to nonluminal subtypes and qRT-PCR signatures for proliferation genes gave more prognostic information than clinical assays

for hormone receptors or Ki67. The authors noted that PAM50 could be a potential replacement for grade-, hormone receptor status, Ki67 index by IHC, and HER2-based prognostic models but not for tumor size and nodal status. Author-noted limitations included the fact that their accounting for pathologic stage was oversimplified and that the population was “strongly biased toward higher-risk breast cancers and likely underestimated the number of patients in broader, node-negative populations for whom adjuvant tamoxifen would represent adequate treatment.”

Parker et al. (2009) published a study in which the PAM50 subtype predictor was developed using qRT-PCR and microarray analysis on 189 breast tumor samples and 29 normal samples from heterogeneously treated patients. A test set of 761 patient who received no adjuvant systemic therapy was used to evaluate prognosis and 133 samples were used to evaluate for pathologic complete response (pCR) to a taxane and anthracycline therapy. Samples included a heterogeneous patient population which was ER, node, and HER2 positive and negative with various tumor sizes, grades and molecular subtypes. The subtypes (i.e., luminal A, luminal B, HER2-enriched, and basal-like) showed and maintained prognostic significance in multivariable analyses incorporating estrogen receptor status, tumor size, node status and histological grade. The subtype model predicted neoadjuvant chemotherapy efficacy with a 97% negative predictive value for pCR. A limitation of the study is the heterogeneity of the patient population and small patient populations.

Randox Assay: The Randox Assay (BCA) (Randox Laboratories Limited, United Kingdoms) is a complementary DNA (cDNA)-based expression biochip assay that is proposed to define clinical sub-types of breast cancer tumors prior to initiating treatment. The target population includes all individuals with a diagnosis of breast cancer (National Institute for Health Research [NIHR], 2011; National Institute for Health and Clinical Excellence [NICE], 2011).

TargetPrint[®]: TargetPrint (Agendia, Irvine, CA) is a microarray for the quantitative assessment of ER, PR and HER2 levels. This diagnostic test measures the expression of ER, PR and HER2 genes at the messenger RNA (mRNA) level compared to the expression of the proteins encoded by the genes as determined by immunohistochemistry (IHC). The test is proposed to be used in conjunction with MammaPrint (Agendia, 2000-2013; Roepman, et al., 2009).

Literature: In the Gevensleben et al. (2010) MammaPrint validation study (n=140) documented above, it was reported that TargetPrint results were validated and concordant with FISH/CISH 97% of the time for ER; 86% for PR; and 94% for HER2. TargetPrint classified two samples as negative that were IHC 2+ for HER2 and not scored by FISH.

Roepman et al. (2009) compared the results of a microarray-based mRNA test (i.e., TargetPrint) to the results of IHC in patients (n=636) with early-stage invasive ductal or invasive lobular breast cancer including two-thirds hormone-positive patients and one-third HER2-positive. ER concordance between TargetPrint and IHC results (n=373) was 93% with 4% of the IHC ER-positive samples being classified as negative by TargetPrint. Eighteen percent of IHC ER-negative tests were classified as ER-positive by TargetPrint. An 83% concordance for PR status and 96% concordance for HER2 status were reported. Nine percent of IHC HER2-positive samples were classified as negative by TargetPrint. Author-noted limitations of the study included that the treatment response information was not available, and that “IHC and microarray rely on the presence of protein or mRNA, but neither assay determines whether that protein or mRNA is capable of making functional receptor proteins so both methods have an uncertainty in predicting whether a tumor is truly positive for functional ER, PR or HER2 protein.” The authors also noted that “conclusions as to which method gives the most valuable readout with respect to patient outcome and treatment response remains to be determined in future studies that include treatment response information for hormone receptor-based treatment (e.g., tamoxifen and ER/PR status) and HER2-targeted therapy (e.g., trastuzumab).

eXagen[™]: eXagenBC (eXagen Diagnostics, Inc., Albuquerque, NM) is a fluorescence in situ hybridization (FISH) assay proposed for assessing breast cancer metastases in women with newly diagnosed, early stage invasive ductal breast cancer. The test has been submitted for FDA approval and is currently only available in investigational use (eXagen, 2009; Ross, et al., 2008).

Additional Assays: Additional gene-profiling assays under investigation include the following:

- Invasiveness Signature[™] (Oncomed Pharmaceuticals, Redwood City, CA) is a test consisting of 186 genes and is designed for node negative, node positive, ER-negative and ER-positive breast cancers.

- NuvoSelect™ eRx 200-gene assay (Nuvera Bioscience, Inc., Woburn, MA) is proposed to predict response to endocrine therapy
- NuvoSelect cRx, a 207-gene predictor is proposed to predict taxane-based chemotherapy response (Nuvera Biosciences, 2012; Ross, 2008; Liu, et al., 2007).
- TheraPrint™ (Agendia, Irvine, CA) is a microarray-based gene assay of 56 genes that has been proposed as potential targets for predicting prognosis and therapeutic response to a variety of therapies. It is still in experimental stages and is used in conjunction with MammaPrint. (Agendia, 2000-2013).

Summary for Other Gene Expression Assays: High-level controlled prospective trial data are limited in the published peer-reviewed literature and accuracy and clinical utility of these assays has not been established. The role of these assays in the management of individuals with breast cancer and their impact on health outcomes is unknown at present.

Systematic Reviews/Multiple Assay Reviews

Nguyen et al. (2012) compared the outcomes of subtyping results using Blueprint, MammaPrint, and TargetPrint to subtyping using immunohistochemistry (IHC)/fluorescence in situ hybridization (FISH) in 132 tumor samples from women with a tumor size ≤ 5 cm, 0 to three positive lymph nodes, and stage T1-4 disease. Blueprint, MammaPrint, and TargetPrint were conducted at Agendia Laboratories and IHC/FISH assessments were conducted at each institution according to their routine practices. IHC/FISH assessed ER, PR and HER2 status (n=132) and Ki-67 (p=79). The concordance between Blueprint and IHC/FISH subtyping was 94% for luminal-type, 95% for HER2-type, and 94% for basal types. The concordance of Blueprint with subtyping using TargetPrint was 96% for luminal type, 97% for HER2, and 98% for basal. The concordance for substratification into luminal A and B using MammaPrint and Ki-67 was 68%. The concordance between TargetPrint and IHC/FISH was 97% for ER, 80% for PR, and 95% for HER2.

Marchionni et al. (2008) conducted a systematic review to summarize studies on Oncotype DX (n=10), MammaPrint (n=4) and H/I expression ratio (n=6). Information was reviewed regarding clinical characteristics of the patients, tumor characteristics, and whether the marketed test or underlying signature was evaluated. The authors noted “the final set of 26 studies included were heterogeneous in focus and quality and that only one study involving Oncotype DX, examined the prediction of treatment benefit. Results from populations that are clinically and therapeutically heterogeneous may not be optimal in determining the prognosis or risk for a particular woman.” “In addition, survival was defined in the studies as disease-free, distant recurrence-free, or overall and over 5 or 10 (or more) years, and prediction strength varied considerably depending on what the test was optimized for. Finally, performance of the underlying gene signature is often close but not identical to the marketed test, because many test procedures, including pretest sample preparation and transport, can differ.” While almost all studies of Oncotype DX used the marketed test as opposed to the signature, the evidence on MammaPrint comes from studies examining the signature and the assay. According to the authors, “The study that compared the results of the marketed MammaPrint test versus its signature on the same samples showed that about 10% of the patients were placed into different risk groups when the marketed test was used. Only 1 seemed to use the marketed H/I test.” “The exact values of the test results provide information that is lost when patients are assigned to risk categories, and the cutoffs for these categories may not correspond to optimal decision thresholds, particularly in combination with other predictors.” How the results of such tests are conveyed to and understood by patients and physicians—for example, as absolute probabilities or as qualitative descriptors (low risk)—is critical because these tests are more widely used. The authors concluded that these technologies “show great promise”, but more information is needed regarding “the extent of improvement in prediction, in whom the tests should be used and how test results are best incorporated into decision making about breast cancer treatment”. They also noted that the “relationship of predicted-observed risk in different populations”, “incremental contribution over conventional predictors, optimal implementation and relevance to patients receiving current therapies” need further study.

Technology Assessments

The Agency for Healthcare Research and Quality ([AHRQ], 2013): On behalf of the AHRQ Raman et al. published a horizon scan titled ‘Update on Emerging Genetic Tests Currently Available for Clinical Use in Common Cancers.’ The assessment provided a brief summary for a number of individual genetic tests for various cancer conditions, including those that may provide prognostic information regarding breast cancer. No recommendations regarding safety, effectiveness, or clinical utility were provided.

The BlueCross BlueShield Association (BCBSA) Technology Evaluation Center (TEC): BCBSA TEC (2010) conducted an assessment to examine “whether, compared to conventional risk assessment tools, the use of gene expression profiling [i.e., Oncotype DX] improves outcomes when used to decide whether risk of recurrence is low enough to forego adjuvant chemotherapy for women with ER-positive, lymph-node-positive breast cancer.” The major study included was the Albain et al. (2010), but it was also noted that the Dowsett et al. (2010) study published after this evaluation, did not affect the results of the Assessment. According to TEC, additional evidence suggested that patients with lymph-node positive cancer treated with chemotherapy were more likely to respond if the RS was high but control arms for comparison were lacking. The Committee stated that there is insufficient evidence to make conclusions regarding the use of Oncotype in node-positive women or the effect of Oncotype on health outcomes in this subpopulation. It has not been demonstrated that Oncotype improves health outcomes in the investigational setting and therefore, it cannot be determined as to whether or not improvement is attainable outside the investigational settings.

In 2008 assessment, BCBSA TEC conducted a technology review on the role of gene expression profiling in the treatment of breast cancer. Included in the report was a review of studies published on Oncotype DX, MammaPrint and Breast Cancer Gene Expression Ratio assays. Regarding Oncotype DX, the report stated that the evidence is sufficient to permit conclusions regarding improved net health outcomes in lymph-node-negative, ER-positive breast cancer patients who met the specific trial enrollment criteria and “provides information about the risk of recurrence that is incremental to conventional classifiers used to predict risk. They also stated that additional studies are needed due to several limitations to the evidence. In relationship to MammaPrint and Breast Cancer Gene Expression Ratio, the report stated that the evidence is insufficient to determine if these assays improve net health outcomes in women with early stage breast cancer.

ECRI: In a 2011 emerging technology assessment, ECRI conducted a systematic review to assess gene expression profiling in predicting treatment benefit from adjuvant chemotherapy/hormonal therapy for patients with early-stage breast cancer. The review included 16 retrospective studies (n=9257). No randomized controlled trials were found. ECRI noted that the studies categorized patients into low- versus high-risk groups or poor-prognosis versus good-prognosis groups and reported a difference in clinical outcomes based on use of the test. Due to the retrospective design of the studies and the potential for biases, firm conclusions on clinical utility could not be made. ECRI made the following conclusions: limited evidence suggested that Oncotype and MammaPrint were able to accurately separate patients into prognostic categories; “the moderate-to-low degree of correlation between the clinical method and gene expression profiling suggests that Oncotype or MammaPrint could be used in conjunction with clinical prognostic tools to further improve accuracy; MammaPrint might be useful in identifying low-risk cases among patients assigned to a higher risk category by Adjuvant! Online”; and no data were available on the use of Oncotype or MammaPrint regarding guiding chemotherapy selection. It was suggested that low-risk patients may not need chemotherapy, whereas, high-risk patients would likely benefit from chemotherapy. The utility of patient’s stratified to Oncotype’s intermediate-recurrence score remained unclear.

Professional Societies/Organizations

American Society of Clinical Oncology (ASCO): In a 2007 update of the recommendations for the use of tumor markers in breast cancer, ASCO stated 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. Oncotype DX may be used to identify patients who are predicted to obtain the most therapeutic benefit from adjuvant tamoxifen and may not require adjuvant chemotherapy”. In addition, patients with high recurrence scores appeared to achieve relatively more benefit from adjuvant chemotherapy than from tamoxifen. They also notes that there was insufficient data to comment on whether these conclusions generalize to hormonal therapies other than tamoxifen, or whether this assay applies to other chemotherapy regimens. The precise clinical utility and appropriate application for other multiparameter assays, such as the MammaPrint assay, the Rotterdam Signature, and the Breast Cancer Gene Expression Ratio are under investigation.

Evaluation of Genomic Applications in Practice and Prevention (EGAPP): The EGAPP Working Group (2009) published recommendations on the use of gene expression profiling for the treatment of women with breast cancer. The review included Oncotype, MammaPrint, and the Breast Cancer Gene Expression Ratio assay. EGAPP concluded that they “found no direct evidence linking tumor gene expression profiling of women with breast cancer to improved outcomes”. Regarding clinical validity, they did state that they “found adequate evidence regarding the association of the Oncotype DX Recurrence Score with disease recurrence and adequate evidence for response to chemotherapy”. Regarding MammaPrint, The EGAPP stated that they found “adequate

evidence to characterize the association of MammaPrint with future metastases, but inadequate evidence to assess the added value to standard risk stratification, and could not determine the population to which the test would best apply". The recommendations stated that these tests have potential for benefit and for harm.

National Cancer Institute ([NCI], 2013): In the Breast Cancer Treatment (PDQ®), the NCI notes "The use of molecular profiling in breast cancer includes the following: ER and PR status testing, HER2/neu receptor status testing, and gene profile testing by microarray assay or reverse transcription-polymerase chain reaction (e.g., MammaPrint, Oncotype DX). No recommendations for safety, effectiveness, or clinical utility were noted.

National Comprehensive Cancer Network (NCCN): In their discussion of prognostic factors for breast cancer, NCCN (2013) stated that the use of DNA microarray technologies has led to the identification of five major subtypes of breast cancer: ER-positive/HER2-negative (luminal A and luminal B); ER-negative/HER2-negative (basal subtype); HER2-positive; and tumors with characteristics similar to normal breast tissue (normal-breast-like). NCCN stated "While many of the DNA microarray technologies are able to stratify patients into prognostic and/or predictive subsets on retrospective analysis, the gene subsets appear to differ from study to study, and prospective clinical trials testing the utility of these techniques have yet to be reported."

Pending the results of the prospective clinical trials, the NCCN Panel considers the 21-gene RT-PCR assay as an option for evaluating primary tumors 0.6–1.0 cm with unfavorable features or > 1 cm and node-negative, hormone-receptor positive, HER2-negative. In this circumstance, the recurrence score may assist in estimating the likelihood of recurrence and benefit from chemotherapy." The NCCN states that the recurrence score "should be used for decision making only in the context of other elements of risk stratification."

NCCN noted that the MammaPrint assay uses microarray technology to analyze a 70-gene expression to aid in the selection of patients with early-stage breast cancer who are likely to develop distant metastasis. NCCN notes that studies using MammaPrint as a prognostic and predictive tool are small and/or retrospective in nature. Multiple other multi-gene or multi-gene expression assay systems that have been developed are "generally based upon small, retrospective studies, and the Panel believes that none are currently sufficiently validated to warrant inclusion in the Guideline. The gene subsets vary from study to study and prospective clinical trials testing the utility of these techniques have yet to be reported". These recommendations are Category 2A: based upon lower level evidence, there is uniform NCCN consensus that the intervention is appropriate.

Use Outside of the US

Several gene expression profiling systems (e.g., OncoType DX, MammaPrint, IHC4, Mammostrat) are being investigated for use outside of the US.

Technology Assessments

National Institute for Health and Care Excellence ([NICE], 2013): In a recently published assessment titled "DG10 Gene expression profiling and expanded immunohistochemistry tests for guiding adjuvant chemotherapy decisions in early breast cancer management: MammaPrint, Oncotype DX, IHC4 and Mammostrat: guidance" NICE noted the following recommendations:

- Oncotype DX is recommended as an option for guiding adjuvant chemotherapy decisions for people with oestrogen receptor positive (ER+), lymph node negative (LN-) and human epidermal growth factor receptor 2 negative (HER2-) early breast cancer if:
 - the person is assessed as being at intermediate risk **and**
 - information on the biological features of the cancer provided by Oncotype DX is likely to help in predicting the course of the disease and would therefore help when making the decision about prescribing chemotherapy **and**
 - the manufacturer provides Oncotype DX to NHS organisations according to the confidential arrangement agreed with NICE.
- NICE encourages further data collection on the use of Oncotype DX in the NHS.
- MammaPrint, IHC4 and Mammostrat are only recommended for use in research in people with ER+, LN- and HER2- early breast cancer, to collect evidence about potentially important clinical outcomes and to determine the ability of the tests to predict the benefit of chemotherapy. The tests are not recommended for general use in these people because of uncertainty about their overall clinical benefit and consequently their cost effectiveness.

National Institute for Health Research (NHS, UK): On behalf of the NHS Ward et al. (2013) published a systematic review of the evidence and cost effective analysis regarding gene expression profiling and expanded immunohistochemistry tests to guide the use of adjuvant chemotherapy in breast cancer management. The objective of this study was to evaluate the clinical and cost-effectiveness of gene expression profiling and expanded immunohistochemistry (IHC) tests compared with existing prognostic tools in guiding the use of adjuvant chemotherapy in women with early breast cancer in England and Wales. Tests using gene expression profiling technology included: Randox Breast Cancer Array, MammaPrint[®], Blueprint[™], the PAM50 gene expression assay, OncotypeDX[™], and the Breast Cancer IndexSM. Outcome measures included analytical validity, clinical validity and clinical utility. Analysis was undertaken for women with ER-positive (ER+), lymph node-negative (N0) and human epidermal growth factor receptor type 2-negative (HER2-) early breast cancer. The tests were assessed as an addition to existing prognostic tools and subgroup analysis was conducted in women with a Nottingham Prognosis Index (NPI) score ≤ 3.4 and score > 3.4 . Thirty-two full-text papers or abstracts representing 30 studies were included in the review. The study populations were generally heterogeneous in the nature of their inclusion criteria although the majority of evidence examined ER+, LN- populations. Most studies included a small number of participants, although a few studies included over 1000 patients. Follow-up was short or not reported for a large number of studies.

The authors noted that “overall Oncotype DX and MammaPrint have a reasonably large evidence base although limitations include heterogeneity of patient populations and retrospective nature of the evidence. Evidence for MammaPrint is primarily observational data of small cohorts, increasing the risk of selection bias.” “Evidence for the clinical utility for these tests is limited by the lack of large prospective studies in UK populations and further evidence is required. PAM50, Blueprint, Breast Cancer Index, NPI+ and Randox Breast Cancer Array have only limited clinical evidence to date.”

OncotypeDX : Twelve additional studies were identified. Previous systematic reviews reported evidence that the OncotypeDX recurrence score was significantly correlated with disease-free survival OncotypeDX was reported to be furthest along the validation pathway. In terms of clinical validity these reviews reported evidence that the OncotypeDX recurrence score was significantly correlated with disease-free-survival and overall survival

MammaPrint: Seven additional studies were identified; none provided evidence of actual changes in treatment decisions following introduction of the test.

PAM50: The NHS evaluated six studies, four were in abstract form. The NHS notes that “the evidence base for PAM50 is still relatively immature. No evidence on clinical utility was identified.”

Mammostrat: The NHS notes “The current review identified three studies that provided data to support the use of the Mammostrat test as an independent prognostic tool for women with ER+, tamoxifen-treated breast cancer. Although the evidence base for the Mammostrat test is relatively immature, these studies included a large sample size, appeared to be of reasonable quality.” The authors reviewed one study clinical utility but noted that limitations were identified relating to this study”

IHC4: Reviewers identified no studies regarding analytical validity. Additionally, no published evidence was identified relative to clinical utility in terms of its impact on treatment decisions or its ability to predict chemotherapy benefit by risk group.

Nottingham Prognostic Index plus, Breast Cancer Index, Blueprint and Randox Breast Cancer Array: The authors noted “No firm conclusions can be drawn about their analytical validity, clinical validity (prognostic ability) and clinical utility. Further evidence on the prognostic and predictive ability of all of these tests is required.”

Ontario Health Technology Advisory Committee (OHTAC): OHTAC (2010) conducted an evidence-based assessment to evaluate the “laboratory performance, prognostic value, and predictive value of Oncotype-DX for women with newly diagnosed early stage I–IIIa invasive breast cancer that was estrogen-receptor (ER) positive and/or progesterone-receptor (PR) positive. However, the review was relevant for women with early stage I and II invasive breast cancer that was ER positive, lymph node negative and HER-2/neu negative. A total of 26 studies met inclusion criteria. The Committee concluded:

1. “There is a lack of external validation to support the reliability of Oncotype-DX; however, the current available evidence derived from internal industry validation studies suggests that Oncotype-DX is reliable (i.e., Oncotype-DX is repeatable and reproducible).
2. Current available evidence suggests a moderate failure rate of Oncotype-DX testing; however, the failure rate observed across clinical trials included in this review is likely inflated; the current Ontario experience suggests an acceptably lower rate of test failure.
3. In women with newly diagnosed early breast cancer (stage I–II) that is estrogen-receptor positive and/or progesterone-receptor positive and lymph-node negative:
 - a. There is low quality evidence that Oncotype-DX has prognostic value in women who are being treated with adjuvant tamoxifen or anastrozole (the latter for postmenopausal women only),
 - b. There is very low quality evidence that Oncotype-DX can predict which women will benefit from adjuvant CMF/MF [cyclophosphamide, methotrexate and fluorouracil 5-FU/methotrexate and fluorouracil 5-FU] chemotherapy in women being treated with adjuvant tamoxifen.
4. In postmenopausal women with newly diagnosed early breast cancer that is estrogen-receptor positive and/or progesterone-receptor positive and lymph-node positive:
 - a. There is low quality evidence that Oncotype-DX has limited prognostic value in women who are being treated with adjuvant tamoxifen or anastrozole,
 - b. There is very low quality evidence that Oncotype-DX has limited predictive value for predicting which women will benefit from adjuvant CAF chemotherapy in women who are being treated with adjuvant tamoxifen.
5. There are methodological and statistical limitations that affect both the generalizability of the current available evidence, as well as the magnitude and statistical strength of the observed effect sizes; in particular:
 - a. Of the major predictive trials, Oncotype-DX scores were only produced for a small subset of women (<40% of the original randomized population) potentially disabling the effects of treatment randomization and opening the possibility of selection bias;
 - b. Data is not specific to HER-2/neu-negative women;
 - c. There were limitations with multivariate statistical analyses.
6. Additional trials of observational design may provide further validation of the prognostic and predictive value of Oncotype-DX; however, it is unlikely that prospective or randomized data will become available in the near future due to ethical, time and resource considerations.
7. There is currently insufficient evidence investigating how Oncotype-DX compares to other known prognostic estimators of risk, such as Adjuvant! Online, and there is insufficient evidence investigating how Oncotype-DX would impact clinician/patient decision-making in a setting generalizable to Ontario”.

Professional Societies/Organizations

European Society for Medical Oncology (ESMO): In their clinical practice guidelines on primary breast cancer, ESMO (2013) noted that “gene expression profiles (e.g., Oncotype and MammaPrint) may be used to gain additional prognostic and/or predictive information to complement pathology assessment and to predict response to adjuvant chemotherapy, especially in ER-positive early breast cancer, however, their true clinical utility is still being evaluated. “ In the 2012 guideline on locally recurrent and metastatic breast cancer, ESMO stated that the “value of multigene assays used for recurrence risk assessment in early breast cancer has not been confirmed in advanced disease”.

IMPAKT (**I**mproving care and **k**nowledge through **t**ranslational research), launched in 2009 by the Breast International Group and the ESMO, in collaboration with a multidisciplinary alliance of European breast cancer organizations and patient groups and is an annual breast cancer conference. Azim et al. (2013) published the IMPAKT 2012 Working Group Consensus statement regarding the utility of prognostic genomic tests in breast cancer practice. A selection of gene expression tests were evaluated, including OncotypeDX and MammaPrint. The authors note “that analytical and clinical validity are convincing for Oncotype DX and MammaPrint; however, the clinical utility is not convincing for any gene expression test that was reviewed. It was not clear from the current evidence that modifying treatment decisions based on the results of a given genomic test could result in improving clinical outcome.”

Summary

When used as a complementary decision-making tool, in combination with other clinical indicators (e.g., tumor size and grade, hormone receptor status, HER2 status), Oncotype DX may provide clinical utility to determine whether or not a specific subset of woman with low-risk indicators might benefit from adjuvant chemotherapy.

Oncotype DX is not indicated as a stand-alone test to be solely relied upon for withholding chemotherapy, nor is it indicated for use in high-risk or intermediate-risk patients (e.g., human epidermal growth factor receptor 2 [HER2]-positive or ER-negative).

The clinical utility and improvements in meaningful health outcomes of other genetic expression assays (e.g., Oncotype DX Breast Cancer Assay for DCIS, Breast Cancer Gene Expression Ratio, HERmark[®] Breast Cancer Assay MammaPrint, Rotterdam Signature 76-Panel) in the treatment of breast cancer have not yet been established through well-designed prospective clinical trials. Studies in the published peer-reviewed scientific literature have primarily been in the form of retrospective validation studies with small heterogeneous patient populations and short-term follow-up. Supporting data on the use of gene expression assays in men with breast cancer are lacking. Studies comparing genetic expression assays to clinicopathologic markers and established algorithm tools are also lacking. The role of such assays is unknown at present.

Coding/Billing Information

Note: 1) This list of codes may not be all-inclusive.

2) Deleted codes and codes which are not effective at the time the service is rendered may not be eligible for reimbursement.

Covered when medically necessary when used to report the Oncotype DX[™] Breast Cancer Assay for women for the specific medical necessity criteria noted above. All other indications and other assays of genetic expression in breast tumor tissue are considered experimental/investigational/unproven and not covered:

CPT ^{®*} Codes	Description
81479	Unlisted molecular pathology procedure
81599	Unlisted multianalyte assay with algorithmic analysis
84999	Unlisted chemistry procedure

HCPCS Codes	Description
S3854	Gene expression profiling panel for use in the management of breast cancer treatment

*Current Procedural Terminology (CPT[®]) ©2012 American Medical Association: Chicago, IL.

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