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**Subject Assays of Genetic Expression
in Tumor Tissue as a
Prognosis in Patients with
Breast Cancer**

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Circulating Tumor Cells Testing
Tumor Markers for Diagnosis and
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Coverage Policy

CIGNA covers Oncotype DX™ 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.
- Breast tumor is estrogen-receptor positive.
- Breast tumor is HER2-receptor negative.
- There is no evidence of metastatic breast cancer, and the patient is axillary-node negative.
- The patient 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 for any other clinical evaluation or any other assays of genetic expression in tumor tissue (e.g., Breast Cancer Gene Expression Ratio, MammaPrint®, Rotterdam Signature 76-Panel) because they are considered experimental, investigational or unproven.

General Background

Breast cancer is a malignant tumor that originates in the breast cells. Types of breast cancers in women include: ductal, lobular, invasive ductal, invasive lobular, phyllodes tumor, tubular carcinoma, mucinous carcinoma, and medullary cancer. Breast cancer can be localized, or it can metastasize to other parts of the body. (American Cancer Society [ACS], 2008; National Comprehensive Cancer Network [NCCN] 2009).

Screening tools for female breast cancer include: breast self-examination, clinical breast examination, and mammography. If the patient 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-receptive- [ER] positive or ER-negative) and the presence or absence of progesterone (i.e., progesterone [PR]-positive or PR-negative), and for the presence of the growth-promoting protein HER2/neu. Breast tumors are also staged according to tumor size (T), lymph node involvement (N) and metastasis (M).

The treatment of breast cancer depends upon the type of cancer (e.g., tumor type and grade, ER-positive/ER-negative, PR-positive/PR-negative, HER2-positive/HER2-negative, invasive/noninvasive); whether or not it has metastasized; patient's age; and comorbidities. Treatment options include: chemotherapy, surgical excision, hormone therapy, immunotherapy, and/or radiation therapy. Chemotherapy may be used as a neoadjuvant therapy prior to surgery to shrink tumor cells, as a primary treatment, or as an adjuvant therapy following surgical excision. Surgical excision may be in the form of a lumpectomy or mastectomy. Monoclonal antibodies are a treatment option for HER2 positive patients. Hormone therapy (e.g., Tamoxifen) is used for patients who are ER-positive (ACS, 2008).

Prognostic factors for recurrence of breast cancer include: tumor size and grade, histological type, hormone receptor status, and lymphatic and/or vascular invasion (Bogaerts, et al., 2006; Lyman and Kuderer, 2006; Andre and Pusztai, 2006). Treatment is based upon these factors, but patients with the same set of factors can have markedly different prognoses. For example, not all patients with larger tumors or positive axillary lymph nodes are destined to progress to metastatic disease, and yet, adjuvant chemotherapy is routinely recommended to nearly all of these patients (Cobleigh, et al., 2005). Considerable differences exist regarding the selection of women who should be treated with adjuvant chemotherapy. It is suspected that patients with ER-positive, lymph node negative (LNN) 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 (Kaklamani, 2006; Lyman and Kuderer, 2006).

Men can also acquire breast cancer. Less than one percent of all breast cancers occur in men and the most common type is infiltrating ductal cancer. The pathology is similar to females. Prognostics factors in men also include the size of the tumor and lymph node involvement. ACS and NCI recommend testing for estrogen- and progesterone-receptor status in men. A small number of breast cancers in men may express the HER2/neu protein. Surgical intervention, and/or radiation therapy and/or hormone therapy and/or chemotherapy are proposed treatment options for male breast cancer (ACS, 2008; NCI, 2008).

Currently, clinicopathologic and immunohistochemistry markers and algorithm tools are used to assist the physicians in predicting the 10-year disease-free and overall survival of patients based upon prognostic factors. These predictions are taken into consideration during the management of breast cancer patients in order to offer the optimal treatment pathways, including the use of hormone and chemotherapy. The likelihood of distant recurrence in patients treated with tamoxifen alone following surgery is estimated at 15% at ten years. At least 85% of women would be over-treated with chemotherapy if it were offered to everyone.

Gene expression analysis has been proposed as an adjuvant tool to assist in determining overall survival, recurrence probability, appropriate treatment options, and responsiveness to hormone and chemotherapies. In gene expression, the total ribonucleic acid (RNA), containing the messenger RNA (mRNA) of expressed genes, is extracted from the tumor tissue, amplified, labeled with a probe, and applied to a silicon chip or glass slide. After appropriate hybridization, the signal for each spot on the chip, corresponding to the abundance of its matching mRNA species, is measured, reflecting the expression level for its gene. A global score for the gene study set can then be identified, and the normalized data allows comparison of the gene expression levels across different tissue specimens. Used in conjunction with consensus guidelines and risk assessments, gene

profiling assays may help to identify those women who do not need adjuvant chemotherapy (NCCN, 2009; ECRI, 2008; Paik, 2006; Calvo, et al., 2005).

Assays of genetic expression in tumor tissue have been proposed as prognostic indicators in the treatment of breast cancer. The Oncotype DX™ 21-gene assay (Genomic Health, Redwood City, CA) has been most widely accepted and recommended for use by the American Society of Clinical Oncologist (ASCO) and by the NCCN. MammaPrint® 70-gene assay (Molecular Profiling Institute (MPI) Inc., Phoenix, AZ and Agendia BV, Amsterdam, The Netherlands) has received FDA approval. Numerous other assays such as the Breast Cancer Gene Expression Ratio (i.e., H/I™) (AviaraDx, Inc., Carlsbad, CA) and the Rotterdam Signature 76-gene panel (Veridex LLC, a Johnson & Johnson Co, Warren, NJ) have also been proposed for use. Some assays are performed in a centralized Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory which means the test does not require FDA approval. Various companies are seeking FDA approval for the assays which allows distribution and use by multiple laboratories. These assays are not advocated as stand-alone tools. They are recommended as an adjuvant tool to be used with other recognized prognostic indicators.

U.S. Food and Drug Administration (FDA)

In February 2007, the FDA approved the first molecular prognostic test, MammaPrint, which is proposed to predict the risk of breast cancer metastasis. MammaPrint is manufactured by Agendia laboratory in the Netherlands (FDA, 2007). The FDA did not require prospective clinical trials for the approval.

Oncotype DX

The purpose of Oncotype DX diagnostic assay is to quantify the likelihood of distant recurrence in a woman with breast cancer, and to aid in assessing the benefit from chemotherapy. The test is recommended to be conducted after the original breast cancer surgery. Using tumor tissue, Oncotype DX assay seeks to quantify the likelihood of recurrence in women with newly diagnosed stage I or stage II, node negative, ER-positive invasive breast cancer, who will be treated with hormone therapy. 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 algorithm was developed based on expression of 16 cancer-related genes identified from 250 gene candidates found in earlier studies and five genes that are included as reference genes. The RS is based on a scale of 0–100. A score of less than 18 is considered low-risk; a score between 18 and 31 is intermediate-risk; and a score over 31 is high-risk. Each RS correlates with a specific likelihood of distant recurrence at 10 years.

The Oncotype DX study (Paik, et al., 2004) used samples from an earlier trial (i.e., National Surgical Adjuvant Breast and Bowel Project [NSABP]) and represented subsets of the overall population. The objective of an ongoing study on “Hormone Therapy With or Without Combination Chemotherapy in Treating Women Who Have Undergone Surgery for Node-Negative Breast Cancer” (TriloraRx), sponsored in part by the National Cancer Institute (NCI), is to validate and extend the Oncotype DX study results (Henry and Hayes, 2006; NCI, 2009).

Literature Review: The studies that have been published on Oncotype included clinical trials that identified and validated the utility of the 21-gene assay and its ability to predict the benefits of chemotherapy in women. Tissue samples were acquired from tumor banks and were from women with various stages and prognostic factors. Supporting data on the use of Oncotype in men and repeat assays after the initial assessment are lacking.

Paik et al. (Aug 2006) conducted a study to determine whether the Oncotype RS predicted the benefit of chemotherapy in NSABP protocol B-20 patients. B-20 protocol included 2363 patients who were randomly assigned to receive tamoxifen or tamoxifen plus fluorouracil or methotrexate and fluorouracil. There were 2299 clinically eligible patients, and paraffin-embedded blocks were available for 651 patients (i.e., 227 tamoxifen and 424 chemotherapy-treated patients). Patients were node-negative, ER-positive and were divided into low- (54.2%), intermediate- (20.6%) and high-risk (25.2%) groups. The magnitude of chemotherapy benefit increased as the RS increased. Patients in this study did not benefit equally from chemotherapy. Patients with tumors who had an RS \geq 31 and had experienced a greater benefit from chemotherapy had a relative risk of 0.26%, and an absolute decrease in 10-year mean distant recurrence rate of 27.6%. An RS $<$ 18 derived little or no benefit from chemotherapy (relative risk of 1.31 and mean absolute decrease in distant recurrence rate of -1.1%). Results for intermediate patients were inconclusive and should not be used to withhold chemotherapy. The test for interaction between chemotherapy and RS was statistically significant. The authors noted that the study

demonstrated that LNN, ER-positive women with tumors with a high RS benefited more from chemotherapy than those with a low RS.

Habel et al. (2006) conducted a case-control study which involved 570 controls and 220 subjects diagnosed with node-negative invasive breast cancer, age less than 75 years, who were not treated with adjuvant chemotherapy, and whose first event was death from breast cancer. Study samples that met inclusion criteria were obtained from a tumor bank of 4964 specimens. Each case was compared to randomly selected matched controls (one to three) that were alive and under follow-up. Adjusting for tumor size and grade, the RS was statistically significant with risk of breast cancer death in ER-positive, tamoxifen-treated and tamoxifen-untreated patients. At 10 years, the relative risk for death in ER-positive, tamoxifen-treated patients was 2.8% for low-risk patients, 10.7% for intermediate-risk patients, and 15.5% for high-risk patients. RS for ER-positive patients not treated with tamoxifen were 6.2% for low-risk patients, 17.8% for intermediate-risk patients and 19.9% for high-risk patients. ER-negative tumors (n=52 cases, 56 controls) reported a relative risk of 6.2%.

Cobleigh et al. (2005) conducted a study to develop the 21-gene RS assay (Oncotype DX) to aid decision-making in relation to chemotherapy in ER-positive, node negative breast cancer patients. RNA was extracted from paraffin blocks; an expression of 203 candidate genes was quantified using RT-PCR. Seventy-eight patients were studied. As of August 2002, 77% of patients had distant recurrence or breast cancer death. Univariate Cox analysis of clinical and immunohistochemistry (IHC) variables indicated that HER2/IHC, number of involved nodes, progesterone receptor (PR)/IHC (%cells), and ER/IHC (%cells) were significantly associated with distant recurrence-free survival (DRFS). Univariate Cox analysis identified 22 genes associated with DRFS. Higher expression correlated with shorter DRFS for the HER2 adaptor GRB7 and the macrophage marker CD68. Higher expression correlated with longer DRFS for tumor protein p53-binding protein 2 (TP53BP2) and the ER axis genes PR and Bcl2. Multivariate methods of the Cox proportional hazards regression model identified several genes, including TP53BP2 and Bcl2, as significant predictors of DRFS.

Esteva et al. (2005) conducted a randomized controlled trial that evaluated the Oncotype DX assay in a group of patients who did not receive adjuvant therapy. These investigators were unable to confirm the results of the Paik et al. (2004) study. Of the 149 patients, 69% were ER-positive. In patients with a low RS, the DRFS rate at 10 years was 82%; in patients with an intermediate RS, the DRFS was 62%; and in patients with a high RS, the DRFS was 78%. There was no significant correlation between DRFS and RS.

Paik et al. (2004) conducted a study to validate the ability of Oncotype DX and the RS to predict the likelihood of distant breast cancer recurrence by evaluating 668 of the 2617 patients enrolled in the tamoxifen arm of the NSABP B-14 trial. In the B-14 trial, patients had been randomized to receive or not receive tamoxifen. The trial was carried out in women with node-negative, ER-positive breast cancer. The primary objective was to determine if there were significantly more patients who were free of distant recurrence for more than 10 years after surgery in the low-risk group than in the high-risk group. In patients with a low RS, the 10-year DRFS was 93.2%; for patients with intermediate RS, the DRFS was 85.7%; and for patients with a high RS, the DRFS was 69.5%. The correlation between DRFS and RS was statistically significant ($p < 0.001$), suggesting that Oncotype DX may help identify tamoxifen-treated, node-negative, ER-positive breast cancer patients with a greater probability of DRFS. The study has a number of limitations.

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. HOXB13 is rarely expressed in normal breast tissue, is expressed in cancerous breast cells and is negatively regulated by estrogen. IL17BR is often lost in the presence of cancer cells and is positively regulated by estrogen. The higher the expression level of HOXB13 and the lower the expression of IL17BR, the greater the chance of tumor recurrence. 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 (Marchionni, et al., 2008; Quest Diagnostics, 2008; Cancer Genetics, 2007; 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: 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. 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 worst 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 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.

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.

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. 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 demonstrated that the H:I ratio is associated with relapse and survival in LNN breast cancer.

Ma et al. (2006) conducted a tumor-bank study to validate the clinical utility of the two-gene ratio demonstrated in the Ma et al. 2004 study. Tissue microarrays were approximately four years old at the time of RNA extraction. Patients were stage I or II primary breast cancer, no distant metastasis, with surgical intervention, with or without radiation therapy, and with or without tamoxifen monotherapy. Nonrelapse cases had a median follow-up of 6.8 years. Expressions of HOXB13, IL17BR, CHDH, ER and PR were quantified using RT-PCR in 852 formalin-fixed, paraffin-embedded tumor specimens, 566 untreated and 286 tamoxifen treated. HOXB13 expression was associated with shorter RFS ($p=0.008$), and IL17BR and CHDC expression were associated with longer RFS ($p < 0.001$; $p < 0.002$, respectively). The H:I ratio predicted clinical outcomes independent of treatment in ER-positive patients, but more so in ER-negative patients. The two-gene ratio was a significant predictor of RFS ($p=0.007$) in ER-positive, LNN subgroup. The study proposed that the H:I ratio is a prognostic factor for ER-positive, LNN patients with or without tamoxifen therapy.

Ma et al. (2004) performed a “genome-wide array analysis of hormone receptor positive invasive breast tumors from 60 patients treated with adjuvant tamoxifen alone therapy” and identified a two-gene expression ratio predictive of clinical outcomes. Available snap-frozen specimens from patients who were ER-positive, early-stage breast cancer, and had a minimum five-year follow up were analyzed. Within a median four years, 28 women developed distant metastasis, while 32 women remained disease-free with a median 10-year follow-up. Using a 22,000 gene oligonucleotide microarray, RNA was isolated from frozen sections from archived primary biopsies. The HOXB13, IL17BR and A1240933 outperformed the growth factor and estrogen and progesterone genes. HOXB13 was overexpressed in tamoxifen recurrence cases and IL17BR was overexpressed in tamoxifen nonrecurrence cases. Further analysis demonstrated that the two-gene ratio had a stronger correlation with treatment outcome than either gene independently. Comparing the H:I ratio to well-established prognostic factors (e.g., age, tumor size, grade, lymph node status), univariate logistic regression analysis indicated only tumor size was significant ($p=0.04$). Compared to tumor size and expression levels of PR gene and ERBB2, the ratio was a highly significant predictor ($p=0.0003$). The study proposed that the H:I ratio is a strong independent predictor of treatment outcomes in setting adjuvant tamoxifen therapy. An analysis was also made by RT-PCR ($n=59$). The RT-PCR data were highly concordant with the microarray data. Twenty additional independent archival cases, ten with recurrence and ten disease-free, were used to demonstrate the predictive utility of H:I ratio and the results demonstrated a high correlation with clinical outcomes ($p=0.024$) with higher HOXB13 expression correlating with poor outcomes. RT-PCR data was used to test the ratio predictability. An overall accuracy of 81%, positive predictive value of 82% and negative predictive values of 82% were reported for the archived biopsies. This model was then applied to the 20 independent-patient group. An overall accuracy of 80%, positive predictive value of 87% and negative predictive value of 75% were obtained. The authors proposed that this gene expression assay could identify patients with ER-positive breast cancer treated with tamoxifen who were at risk for tumor recurrence, but noted that the study was limited by its size and confirmation in a larger population was warranted.

MammaPrint

MammaPrint was originally developed at the Netherlands Cancer Institute by the Molecular Profiling Institute, Inc. (MPI). MPI was developed by two nonprofit organizations, the Translational Genomics Research Institute (TGen, Phoenix, AZ) and the International Genomics Consortium (IGC, Phoenix, AZ). MammaPrint is a deoxyribonucleic acid (DNA) microarray assay used to assess breast tumors ≤ 5 centimeters in patients less than age 61 who have stage I, invasive breast cancer with ER-positive or ER-negative receptors, or stage II invasive breast cancer, with ER-positive or ER-negative receptors, and are lymph node negative. 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 profile are used to predict the risk of metastasis (Agendia, 2008). Currently, MammaPrint is only performed in the Agendia laboratory and all samples must be shipped to the Netherlands (BlueCross BlueShield Association [BCBSA], 2007).

The European Organization for Research and Treatment of Cancer (EORTC) is conducting a multicenter, prospective phase III, randomized study (**Microarray In Node negative Disease may Avoid ChemoTherapy [MINDACT]**) to “compare 70-gene expression with common-clinical pathological criteria in selecting patients for adjuvant chemotherapy in node-negative breast cancer.” The trial will compare the 70-gene assay to the Adjuvant! Online, a clinical-pathological prognostic tool used in selecting patients for adjuvant chemotherapy who have node-negative breast cancer (Bertucci, et al., 2006; EORTC, 2006).

Literature Review: Because MammaPrint was designed to identify younger patients who are at risk for distant metastasis, Wittner et al. (2008) conducted a retrospective analysis of the negative predictive value (NPV) and positive predictive value (PPV) of MammaPrint in 100 older women (69 women were age 55 years or older; median age 62.5 years). Samples taken from a tumor bank included those of women who were consecutively diagnosed and treated for lymph node–negative, invasive breast cancer with predominately small and low-grade tumors. Subjects were treated with surgery and/or radiation therapy or with chemotherapy and/or hormonal therapy. Follow-up ranged from 1.2–13.0 years. Twenty-seven women were classified as low-risk and 73 as high-risk for recurrence. There was no statistically significant difference in time to distant metastasis between low-risk and high-risk patients. The NPV was 100% for women at low-risk for distant metastases at five years. Nine patients who developed distant metastases as a first event had primary tumors that were rated as high-risk. The gene test classified 64 women as having a poor prognosis who were actually low risk making the PPV 12% for distant metastases at five years. A range of alternative cutoffs for analysis of PPV did not reveal any analysis that could yield a high PPV. Used in conjunction with the Adjuvant! Online tool, an additional 26 patients were classified as low risk and those women did not develop distant metastasis as a first event.

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. 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.

Buyse et al. (2006) described the results of a TRANSBIG collaborative study intended to validate the 70-gene MammaPrint signature in a group of independent breast cancer patients. The aim of the study was “to determine if the gene signature had prognostic value independent of the clinical risk classifications”. Subjects included 302 patients from five centers who were divided into high-risk and low-risk groups based on gene signature and clinical risk classifications. Frozen specimens were sent from the clinical areas to the Netherlands Cancer Institute for analysis. Inclusion criteria incorporated patients who were diagnosed before 1999 at age < 60 years, LNN, ≤ 5 centimeter (cm) tumor, and had not been treated with adjuvant systemic therapy. Median follow-up was 13.6 years. From time to distant metastases, the gene signature hazard ratio (HR) was 2.32 without adjustment for the clinical risk, and the unadjusted clinical pathologic risk by Adjuvant! Software was 1.68. For overall survival, the gene signature high-risk group unadjusted HR was 2.79, while the clinicopathologic unadjusted rate was 1.67. The high-risk gene signature group 10-year survival rate was 0.69 for both the low- and high-risk clinical groups. The low-risk gene signature group 10-year survival rate was 0.88 for the low-risk clinical group and 0.89 for the high-risk clinical group.

In 2006, Glas et al. published a study that converted the 70-gene expression profile identified by Van De Vijver et al. (2002) into the customized microarray, MammaPrint, which contained a reduced set of 1900 probes suitable for processing. Patients (n=151) from the original cohort (n=295) who were LNN yielded 145 RNAs for comparison. The probability of a patient remaining free of distant metastases and overall survival were calculated. The correlation between the original data and the array from this study were highly similar (p<0.0001).

Van De Vijver et al. (2002) conducted a study using microarray analysis to evaluate a previously established 70-gene prognosis profile. A series of 295 consecutive patients with primary breast carcinomas were selected as having a gene expression signature associated with either a poor or a good prognosis. Tumors were selected from the fresh-frozen–tissue bank of the Netherlands Cancer Institute. All patients had stage I or stage II breast cancer, were younger than age 53, and were LNN (n=151), or lymph node positive (n=144). The predictive

power of the prognosis profile using univariable and multivariable statistical analyses was evaluated. Among the 295 patients, 180 had a poor prognosis signature and 115 had a good prognosis signature. The mean overall survival rates at 10 years were 54.6% and 94.5%, respectively. At 10 years, the probability of remaining free of distant metastases was 50.6% in the poor prognosis signature group and 85.2% in the good prognosis signature group. The estimated HR for distant metastases in the group with a poor prognosis signature, as compared to the group with the good prognosis signature was 5.1. This ratio remained significant when the groups were analyzed according to lymph node status.

It is yet to be proven in prospective, clinical trials that MammaPrint improves stratification beyond what is currently available through clinical and histopathological assessment.

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. This is the first proposed assay that represents a prognostic molecular marker that could be used with all lymph node negative (LNN) breast cancer patients, regardless of age, tumor size and grade, or ER status. Microarray technology allows researchers to study thousands of genes at one time or select only those genes that are believed to be implicated in a particular cancer. Analysis of the 76-gene signature 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: 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) for OS. In the low genomic and low clinical risk groups, no patient developed distant metastasis.

Foekens et al. (2006) conducted an independent validation study of the 76-gene expression assay by analyzing 180 LNN, frozen samples from four European institutions. Inclusion criteria included: patients who had not received adjuvant systemic treatment; patients with more than five years follow-up without distant relapse within those five years; and patients who had demonstrated no evidence of recurrent disease within one month following initial surgical treatment. Follow-up of the subjects ranged from 48–137 months. The actuarial five-year distant metastasis-free survival rates were 96% for the good profile groups and 74% for the poor profile group, and the 10-year rates were 94% for the good profile group and 65% for the poor profile group. The five-year distant metastasis survival rate was 90% with 50% specificity. The positive predictive value was 38%, and the negative predictive value was 94%. In a subset of ER-positive patients (n=55), LNN patients treated with tamoxifen, the hazard ratio (HR) was 6.15, with a sensitivity of 80% and a specificity of 40% for distant metastasis-free survival.

The original study that identified the 76-gene signature assay was conducted by Wang et al. (2005) using frozen tumor samples from a tumor bank in Rotterdam, The Netherlands, which received samples from 25 regional hospitals. The samples were from LNN breast cancer patients, treated from 1980–1995, who had not received neoadjuvant or adjuvant therapy. Patient age range at the time of the initial surgical treatment was 26–83 years. Follow-up ranged from 20–171 months. There were no significant differences between the groups in menopausal status. ER-negative patients had a higher proportion of larger tumors and more poor-grade tumors than ER-positive patients. Patients were randomly divided into a training set and a testing set. Expression

values were calculated using the Affymetrix Human U133a GeneChip® (Affymetrix Inc., Santa Clara, CA). A sample of 286 tumors provided 17,819 genes for hierarchical clustering. From a training set of 115 tumors, the study identified a 76-gene signature, which included 60 genes from ER-positive patients and 16 genes from ER-negative patients. Ninety-three percent sensitivity and 48% specificity were demonstrated in an independent testing of 171 LNN patients. Patients who developed distant metastases within five years were identified with an HR of 5.67. The authors noted that this assay could be applied to all LNN patients, pre- and postmenopausal, and patients with tumors 10–20 mm in size.

The published peer-reviewed scientific literature is limited and does not support the clinical utility of the Rotterdam signature test. There is a lack of published, prospective studies demonstrating the clinical utility of this gene signature.

Other Gene Assays

Breast BioClassifier™: Breast BioClassifier (AURA, Salt Lake City, UT) is a 55-gene (i.e., 50 classifier genes and five control genes) RT-PCR assay that classifies ER-positive and ER-negative breast cancers to help predict outcomes. The test provides biologic subtypes of the cancers and reports outcomes on as a continuous risk score. The company is waiting FDA approval (ARUP, 2009; Ross, et al., 2008).

Breast Cancer Gene Expression Prognosis Profile: Breast Cancer Gene Expression Prognosis Profile is a 14-gene signature proposed for use in lymph node negative, ER-positive patients to estimate the likelihood of recurrence including distant metastasis.

Using formalin-fixed embedded tissue samples, Tutt et al. (2008) identified a prognostic gene-expression signature in women with operable, invasive ER-positive, lymph node negative breast cancer. Patients had received no systemic therapy following surgery. Using 197 genes from previously identified gene signatures (e.g., MammaPrint and Oncotype), the authors identified the 14-gene signature used in this study. The training set specimens were obtained from 142 patients with T1 and T2 breast cancer who met inclusion criteria. Patients were followed for eight or more years, median follow-up 14.8 years. The primary endpoint was the time from surgery to distant metastasis. The time from surgery to death was analyzed as overall survival. The 14 genes were found to be prognostic and were used to generate a metastasis score (MS). A significant association ($p < 0.05$) was found between the 14-gene assay and distant metastasis. A retrospective validation study ($n = 279$) was then conducted on independent tumor samples from women with an ER-positive, ≤ 3 cm tumor who were lymph node negative. The median follow-up was 15.6 years. In the validation test, the MS was associated with as statistically significant predictor of distant metastasis ($p < 0.0001$) and overall survival ($p = 0.0004$). The sensitivity and specificity of the validation 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 validation MS risk groups to predict death from any cause at 10 years were 84% and 45%, respectively. The study also analyzed the predictive ability of the 14-gene signature in 45 node-negative, ER-positive, T1 patients treated with tamoxifen. The signature predicted two risk groups, but due to the small sample size the authors noted that a larger data set would be required to discern the prognostic ability of the 14-gene signature in tamoxifen-treated patients. Limitations noted by the author included the retrospective study design and the fact that the study population may not represent ER-positive patients today because the samples were collected (1975–1986) before tamoxifen became a routine treatment option for patients.

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, 2008; Ross, et al., 2008).

Mammostrat®: Mammostrat (Applied Genomics, Inc. [AGI], Huntsville, AL) is a prognostic IHC test that uses five monoclonal antibody biomarkers to detect gene expression of proteins. 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. AGI is working to obtain FDA approval (AGI; Ross, et al., 2008; Ring, et al., 2006).

Theros Breast Cancer IndexSM: Theros Breast Cancer Index (bioTheranostics, San Diego, CA) is a combination of the Theros H/ISM and the bioTheros MGISM. The Theros H/I is a two-gene index that stratifies ER-

positive cancer for endocrine therapy benefit. The Theros MGI is a five-gene index that provides quantitative and objective molecular assessment of tumor grade and proliferative rate. MGI discriminates between tumor grades 1 and 3 and reclassifies grade 2 tumors into low- or high-risk. It is also a prognostic indicator for ER-positive patients regardless of nodal status and indicative of response to chemotherapy. Each test stratifies breast cancers as low or high for recurrence. It is proposed that by combining these two individual test results, one would obtain independent and complementary prognostic information. 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 is a CLIA-certified laboratory (bioTheranostics, 2009).

Additional gene-profiling assays under investigation include the Invasiveness Signature™ (Oncomed Pharmaceuticals, Redwood City, CA). This test consists of 186 genes and is designed for node negative, node positive, ER-negative and ER-positive breast cancers. Nuvera Bioscience, Inc., (Woburn, MA) is developing the NuvoSelect™ eRx 200-gene assay to predict response to endocrine therapy and the NuvoSelect cRx, a 207-gene predictor of taxane-based chemotherapy response (Ross, 2008; Nuvera Biosciences, 2008; Liu, et al., 2007).

Systematic Reviews/Technology Assessments

ECRI Institute (2008) conducted a systematic review of the literature and concluded “existing studies provide clinical validation for the ability of the Oncotype DX assay and the MammaPrint assay to predict tumor recurrence and response to chemotherapy. However, the studies are insufficient to allow one to draw strong conclusions regarding the clinical utility of these assays for guiding treatment decisions for patients with early-stage invasive breast cancer”.

Marchionni et al. (2008) conducted a systematic review to summarize studies published from 1990 through January 2007 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 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.

The BlueCross BlueShield Association Technology Evaluation Center (TEC) published a TEC Assessment on the role of gene expression profiling in the treatment of breast cancer. Included in the report was a review of studies published from January 2005 through December 2007 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 breast cancer patients. “Epidemiologic analyses show that Oncotype DX™ RS is strongly and independently associated with the risk of distant recurrence or death from breast cancer in patients with lymph-node-negative, ER-positive breast cancer who were treated with tamoxifen and who met other specific trial enrollment criteria,” and “provides information about the risk of recurrence that is incremental to conventional classifiers used to predict risk.” The use of Oncotype DX is “likely to change the therapy decisions a patient and her physician would otherwise make using conventional-risk classifiers”. They also state that additional studies are needed due to several limitations to the evidence. In relationship to MammaPrint and Breast Cancer Gene Expression Ratio, the TEC states that the evidence is insufficient to determine if these assays improve net health outcomes in women with early stage breast cancer (BCBSA TEC, 2008).

Lyman and Kuderer (2006) conducted a systematic review, including a meta-analysis, of all-language published studies through 2006 on gene expression profiling (i.e., 76-gene, 70-gene, 64-gene 41-gene; 23-gene, and 21-gene expression profiles) in patients with early-stage breast cancer. Out of 587 articles, 17 separate cohorts of patients were identified which reported a gene expression signature in relationship to distant recurrence-free survival. The studies included 20–668 patients each (2908 total), of which 1531 were classified as high-risk based upon gene assay, with 595 experiencing distant recurrence. Median follow-up ranged from 2–14 years. Adjuvant treatment varied among the studies (i.e., no treatment, tamoxifen only, chemotherapy, or treatment was not specified). Studies also varied with a mixture of node-negative or node-positive or both negative and positive disease. Four studies were limited to hormone receptor-positive patients. The false-positive rate was > 50% in six studies, and the false-negative rate was > 20% in eight studies. Substantial heterogeneity was

seen across studies, and some studies were based on cross-validation, which may overestimate the accuracy of the assay. Independent validation of a completely separate patient population offers the optimal approach. While the overall sensitivity was high (80.6%), the overall specificity of this analysis was quite low (53.6%). The authors noted that gene assays show promise for predicting survival, but “the use of these assays in therapeutic decision-making must consider the limitation of assay test performance and the specific patient population being evaluated”.

Professional Societies/Organizations

The Evaluation of Genomic Applications in Practice and Prevention (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.

In a 2007 “update of the recommendations for the use of tumor markers in breast cancer”, the American Society of Clinical Oncology (ASCO) states 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 appear to achieve relatively more benefit from adjuvant chemotherapy (specifically (C)MF) than from tamoxifen. There are insufficient data at present 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 (Harris, et al., 2007).

The National Comprehensive Cancer Network (NCCN) discusses the use of gene expression profiling in the management of breast cancer patients and proposes that this technology will play an important role as a prognostic tool in the future. NCCN states “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 TAILORx and MINDACT clinical trials, the NCCN Panel considers Oncotype DX as an option for evaluating “primary tumors characterized as 0.6–1.0 cm with unfavorable features or > 1 cm and node-negative, hormone-receptor positive and HER2-negative. In this circumstance, the recurrence score may assist in estimating the likelihood of recurrence and benefit from chemotherapy.” They stress that the recurrence score should be used “for decision making only in the context of other elements of risk stratification”. These recommendations are based upon nonuniform NCCN consensus based on lower-level evidence, including clinical experience (NCCN, 2009).

In their discussion of the 70-gene, 76-gene and 21-gene expression assays, the National Breast Cancer Coalition (NBCC) (2007) states that “published research to date is very limited, and further clinical research will be needed to determine whether such tests will be useful in helping women make treatment decisions that are clinically sound. Prospective, randomized clinical trials are needed to determine the clinical relevance of these tests and their value relative to traditional prognostic and predictive methods.” NBCC states that, although they do not endorse specific drugs, devices, or procedures for breast cancer care, they are excited about gene expression profile tests, but these tests must be “appropriately validated and studies replicated before they are used in the clinic outside of research protocols.” They encourage women to participate in well-designed, controlled clinical trials.

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 woman with low-risk indicators (e.g., tumor size \leq 1 centimeters, tumor grade 1 and estrogen-receptor positive) 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 of other genetic expression assays (e.g., Breast Cancer Gene Expression Ratio, MammaPrint, Rotterdam Signature 76-Panel) in the treatment of breast cancer has not yet been established through well-designed clinical trials. Supporting data on the use of gene expression assays in men and repeat assays after the initial assessment are lacking.

Coding/Billing Information

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

Covered when medically necessary:

CPT ^{®*} Codes	Description
	Multiple/Varied

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

[†]**Note:** Covered when used to report the Oncotype DX[™] assay 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 or unproven and are not covered.

ICD-9-CM Diagnosis Codes	Description
174.0-174.9	Malignant neoplasm of female breast
233.0	Carcinoma in situ of breast

*Current Procedural Terminology (CPT[®]) © 2008 American Medical Association: Chicago, IL.

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Policy History

Pre-Merger Organizations	Last Review Date	Policy Number	Title
CIGNA HealthCare	3/15/2008	0298	Assays of Genetic Expression in Tumor Tissue as a Prognosis in Patients with Breast Cancer
Great-West Healthcare	11/30/07	05.303.02	Genetic Testing, Breast Cancer Assay of Genetic Expression

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Connecticut General Life Insurance Company has acquired the business of Great-West Healthcare from Great-West Life & Annuity Insurance Company (GWLA). Certain products continue to be provided by GWLA (Life, Accident and Disability, and Excess Loss). GWLA is not licensed to do business in New York. In New York, these products are sold by GWLA's subsidiary, First Great-West Life & Annuity Insurance Company, White Plains, N.Y.