

TITLE: The 70-Gene Signature (MammaPrint) As A Guide For The Management Of Early Stage Breast Cancer

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THE 70-GENE SIGNATURE (MAMMAPRINT) AS A GUIDE FOR THE MANAGEMENT OF EARLY STAGE BREAST CANCER

A Technology Assessment

INTRODUCTION

Profiling the patterns of gene expression for an individual breast tumor has the potential to choose therapy based on the specific genetic characteristics of the individual's tumor. The California Technology Assessment Forum (CTAF) reviewed the scientific literature on the safety and efficacy of gene expression profiling for the management of early stage breast cancer in October 2006 and found that TA criteria 1 through 5 were met for the 21-gene recurrence score (OncotypeDX®). Since that time, both the American Society for Clinical Oncology and the National Comprehensive Cancer Network (NCCN) have updated their guidelines to recommend the use of the 21-gene recurrence score assay to guide therapy for early breast cancer. The California Technology Assessment Forum has been asked to update their review focusing on the evidence for the safety and efficacy of the 70-gene signature (MammaPrint®).

BACKGROUND

Breast Cancer

Cancer of the breast is the most common form of cancer in women. Every American woman is estimated to have a one in eight chance of developing breast cancer at some time during her life. In 2009, there were an estimated 194,280 new cases of invasive breast cancer in the United States and an estimated 40,610 deaths from this cancer. This represents approximately 27% of all new cancer cases and 15% of all cancer deaths in women.¹

The staging system of the American Joint Committee on Cancer defines early stage (Stage I, II, and IIIA) invasive breast cancer as tumors ≤ 5 cm in largest dimension, with up to three positive axillary nodes and without distant metastasis or involvement of the fixed axillary or internal mammary lymph nodes (T1-2, N0-1, M0). In the TNM (Tumor Node Metastasis) staging system for breast cancer, stage T1 refers to carcinomas 2.0 cm or less in greatest dimension; stage T2 refers to tumors more than 2.0 cm, but not more than 5.0 cm



in greatest dimension; stage T3, to tumors more than 5.0 cm in greatest dimension; and stage T4, to tumors with direct extension to chest wall or skin. Stage N0 refers to tumors without regional lymph node metastasis and N1 refers to tumors with ipsilateral metastases to one to three axillary lymph nodes. N2 refers to involvement of four or more axillary nodes. M0 refers to tumors without distant metastases.

- *Stage 0* - Carcinoma in situ
- *Stage I* - Tumor (T) does not exceed 2 cm, no axillary lymph nodes (N) involved.
- *Stage IIA* – T 2-5 cm, N negative, or T <2 cm and N positive.
- *Stage IIB* – T > 5 cm, N negative, or T 2-5 cm and N positive (< 4 axillary nodes).
- *Stage IIIA* – T > 5 cm, N positive, or T 2-5 cm with 4 or more axillary nodes
- *Stage IIIB* – T has penetrated chest wall or skin, and may have spread to < 10 axillary N
- *Stage IIIC* – T has > 10 axillary N, 1 or more supraclavicular or infraclavicular N, or internal mammary N.
- *Stage IV* – Distant metastasis (M)

Chemotherapy in early stage breast cancer

The NCCN recommends that chemotherapy be offered to patients with breast cancer based on tumor stage, hormone receptor status, and human epidermal growth factor receptor (HER2) status.² They recommend against chemotherapy for women with small (≤ 0.5 cm or grade 1 tumors 0.5-1 cm in size), hormone receptor positive tumors. For women with estrogen receptor (ER)-positive tumors greater than one cm in diameter they recommend considering the 21-gene recurrence score and not offering chemotherapy to women with a low recurrence score (<18). Endocrine therapy alone is sufficient in these patients. The most recent St. Gallen International Expert Consensus conference on the primary therapy of early breast cancer met in 2009.³ Their recommendations are similar to those of the NCCN, but allowed for the use of any “validated multigene test” to guide decision-making about chemotherapy in women with hormone receptor positive tumors. National Cancer Institute (NCI) treatment guidelines recommend that adjuvant chemotherapy be offered to all women with Stage I breast cancers > 1 cm in diameter, but not for women with hormone receptor positive, grade 1 tumors less than 1 cm. They do not comment on gene expression profiling. A different approach is used by Adjuvant!, a free computer program used by many oncologists when counseling patients.⁴ The program uses the patient’s age, morbidity, receptor status, tumor size and grade, and number of positive lymph nodes to calculate her ten year risk of relapse and death based on data from large randomized trials and the SEER registry. The numbers can be recalculated to assess the effect of hormone therapy, chemotherapy, and combined therapy. Decisions are then made based on the patient’s personal values of the absolute magnitude of the benefit compared with the harms of chemotherapy.



Staging and risk assessment have important limitations. Patients with the same stage breast cancer can have markedly different responses to therapy and long-term outcomes.^{5,6} The majority of women with hormone receptor positive, node negative breast cancer have no evidence of breast cancer recurrence after ten years of follow-up, even if they are classified in a higher risk group using standard clinical measurements. In the NSABP-20, a randomized trial of women with estrogen receptor positive, lymph node negative breast cancer less than five cm in diameter, 83% of women treated with tamoxifen alone were disease free at ten years.⁷ However, many patients with early stage breast cancer are treated with chemotherapy in the United States. Thus, it has long been hypothesized that many of the patients with early stage breast cancer who are treated with chemotherapy derive no benefit from the treatment, yet receive all of the treatment related adverse effects. One of the driving forces behind the characterization of tumors with gene expression profiling is to better define which patients will benefit from chemotherapy and which will not. Additionally, some of the patients currently identified as low risk may benefit from chemotherapy. Tools such as Adjuvant! assess risk for populations, rather than for the individual. It is the hope that evaluating individual tumor biology will lead to personalization of cancer therapy to maximize efficacy and minimize toxicity.

Gene expression profiling

Gene expression profiling refers to a number of different technologies that attempt to quantify the relative levels of messenger RNA (mRNA) for large numbers of genes in specific cells or tissues. The goal is to measure differences in the level of translation (expression) of different genes and utilize patterns of differential gene expression in order to characterize different biological states of the tissue. This allows for the simultaneous evaluation of thousands of markers and their associated patterns, rather than evaluating them individually, as has traditionally been done. In addition, gene expression is an accurate and powerful way to evaluate differences in markers known to correlate with response and outcome, such as the estrogen and progesterone receptors. Another potential value of this approach is the identification of genes and gene products associated with a disease process that were not previously known. In cancer biology, the technology has been used to try to differentiate between different subtypes of cancers⁸⁻¹⁴, to identify tumors with good and bad prognoses^{9, 14-21}, and to identify subgroups of tumors with a high likelihood of responding to one therapeutic regimen compared with another.^{22, 23}

Ribonucleic acid (RNA) is rapidly broken down in tissue samples. Thus, gene expression profiling generally requires fresh tissue that has been immediately processed to isolate and stabilize the RNA content or the



tissue must be immediately frozen for later processing. The most common approach to gene expression profiling utilizes arrays of deoxyribonucleic acid (DNA) sequences bound to a surface like a glass slide. Often tens of thousands of DNA sequences are organized on an individual microarray in an attempt to profile all of the 20-30,000 genes in the human genome. RNA is isolated from a test sample (tumor, white blood cells, normal tissue), amplified, and labeled with a fluorescent dye. Then it is exposed to the surface of the microarray to allow hybridization with the DNA spots bound to the microarray surface. Usually the hybridization occurs with a mixture of RNA from a control sample labeled with a second fluorescent dye. Any sample RNA that matches DNA on the microarray (complementary sequences) is bound to the microarray at a specific location. The remaining sample is then washed away. The amount of DNA binding at each site is measured by the intensity of the fluorescent signal. Since the identity of the DNA at each site on the microarray is known, the degree of fluorescence can be correlated with the relative amount of RNA in the original sample. Many genes (“housekeeping” genes: genes that tend to be transcribed continuously at a relatively constant level and usually function to maintain the cell) are expressed at the same level as the control DNA. The genes of interest are those that are over- or underexpressed relative to the control DNA.

Another approach to the measurement of gene expression is real-time, reverse-transcriptase polymerase chain reaction (RT-PCR). This approach uses the reverse transcriptase enzyme to generate complementary DNA (cDNA) from the mRNA in a sample. The cDNA is then amplified using PCR. RT-PCR is more reproducible and quantitative than gene profiling with expression arrays.^{24, 25} In particular, the precision and dynamic range of RT-PCR is greater than that of gene expression arrays. Initial discovery is often done with expression arrays, but once the discriminatory genes have been identified, RT-PCR is often used to quantify the relative amounts of a smaller set of genes. This test has the additional advantage that it can be performed on paraffin embedded tumor tissue, when a limited number of genes are analyzed.

Gene expression experiments usually start with microarrays containing many thousands of genes and compare the profiles of tissue with and without certain characteristics in order to identify a smaller subset of genes that differentiate between the two states (rejection/no rejection; metastases/no metastases). This smaller subset of genes is then validated using new patient samples. Additional candidate genes based on known biological associations may also be included.

These experiments generate tens of thousands of data points, but microarrays are expensive. Appropriate tissue from patients with outcomes of interest is limited, so the number of patients evaluated in microarray experiments is often quite low. Much has been written about the statistical dangers of evaluating thousands



of predictor variables in small datasets (multiple hypothesis testing, overfitting).²⁶⁻²⁸ It is essential that any pattern identified by such experiments be independently validated. Unfortunately, excitement about the results from initial experiments has often overwhelmed statistical caution. One recent paper re-evaluated the data from seven gene expression profiles of cancer prognosis and showed that five of them were likely to predict outcome no better than chance.²⁹ Ideally, results from a gene expression profile should, at a minimum, be validated in a new set of patients by a group of investigators independent from those initially developing the test. Validation means re-evaluating the test characteristics of the same assay in a new set of patients.

Gene expression profiles of breast tumors were initially analyzed with unsupervised clustering, which groups sets of cancers together based on similar patterns of DNA expression.³⁰ These intrinsic gene patterns identified five subtypes of breast cancer that apparently were subsequently found to have prognostic, as well as biological, implications.^{12, 31, 32} That spawned a series of gene expression array experiments focused on improving risk prediction based on RNA expression levels in the primary breast tumors. Two commercial tests based on these experiments, MammaPrint® and Oncotype DX, are currently marketed to physicians treating breast cancer. This is likely the tip of the iceberg: as preservation of tumor RNA from tumors becomes standard and technology improves, novel profiles and refinements of the existing profiles will be developed and commercialized.

MammaPrint / The Amsterdam Profile / The 70-gene prognostic signature

MammaPrint is a custom microarray chip designed to assay the mRNA expression of 70 genes in triplicate. It requires either fresh or snap-frozen tissue. These 70 genes were identified in one of the first gene expression profiling experiments performed explicitly to find gene patterns that differentiated tumors likely to metastasize from tumors likely to be cured with local therapy alone.²¹ It is sometimes known as the Amsterdam profile because it was developed by scientists at the Netherlands Cancer Institute (NKI) in Amsterdam. The pattern was developed in patients under the age of 55 years with lymph node negative breast cancers (N0) and the commercial test is marketed for use in this population irrespective of ER status. Overexpressed genes in the profile are involved with cell cycle regulation, angiogenesis, invasion, cell migration, and signal transduction. The test result is binary, either good prognosis or poor prognosis, based on the correlation of the 70 genes with the profile identified in the initial study.



Technology Assessment (TA)

TA Criterion 1: The technology must have the appropriate regulatory approval.

Until recently Oncotype DX, the Amsterdam signature, and other Gene Expression Profiles were considered Home Brew tests and exempted from FDA oversight. However, on September 7, 2006, the US Food and Drug Administration (FDA) published draft guidance on planned regulation of In Vitro Diagnostic Multivariate Assays (IVDMIA). Complex tests combining data from multiple laboratory tests using a complex algorithm, like those derived from gene expression profiles, will be subject to FDA review in the future. MammaPrint received FDA approval via the IVDMIA mechanism on February 6, 2007 through the 510(k) process as a Class II device with special controls for use as a prognostic test for breast cancer patients less than 61 years old with stage I or II, with tumor size ≤ 5.0 cm and who are node negative. The MammaPrint result is indicated for use by physicians as a prognostic marker only, along with other clinicopathological factors. The FDA also noted the following special condition: "MammaPrint® is not intended for diagnosis, or to predict or detect response to therapy, or to help select the optimal therapy for patients." It was the first test to be approved as an IVDMIA device.

TA Criterion 1 is met.

TA Criterion 2: The scientific evidence must permit conclusions concerning the effectiveness of the technology regarding health outcomes. For diagnostic tests, there is evidence that use of the test would result in improved medical management in a way that will benefit the patient.

The Medline database, Cochrane clinical trials database, Cochrane reviews database and the Database of Abstracts of Reviews of Effects (DARE) were searched using the key words 'breast neoplasms' and 'gene expression profiling'. These were cross-referenced with the keyword 'human'. The search was performed for the period from January 2006 through May 2010 to update the prior review. The search identified 2558 articles. The bibliographies of systematic reviews and key articles were manually searched for additional references. References were also solicited from the manufacturer and local experts. The abstracts of citations were reviewed for relevance and all potentially relevant articles ($n=47$) were reviewed in full. In order to be included in this systematic review, articles had to compare the five year outcomes for women with breast cancer for high and low risk groups identified by the gene expression profile. Ideally, the study



would evaluate the incremental benefit of gene expression profiling over standard risk assessment tools or guidelines such as Adjuvant!⁴ Online or the St. Gallen Consensus Guidelines. As noted above, this review will focus on the 70-gene prognostic signature developed by Dr. van't Veer and colleagues.^{20, 21, 33-46}

None of the studies with clinical outcomes used the gene expression profile to guide patient management. Ideally, randomized clinical trials would compare the clinical outcomes of patients with early stage breast cancer managed using standard risk assessment to those of patients managed using information from gene expression profiling. One such randomized trial is currently accruing patients, but will require at least five years to report preliminary outcomes. The trial is directly comparing care guided by a gene expression profile to care guided by clinical criteria when the two conflict. One retrospective study, described below, compares the outcomes of patients treated with chemotherapy to those not treated with chemotherapy within the risk groups defined by MammaPrint to evaluate whether it may be useful as a predictive test in addition to its value as a prognostic test.⁴²

Level of evidence: 3-5

TA Criterion 2 is met.

TA Criterion 3: The technology must improve the net health outcomes.

In oncology, clinicians and researchers make a distinction between prognostic factors and predictive factors. More than 100 prognostic factors have been described as risk factors for breast cancer recurrence.⁴⁷ However, only a few of these risk factors are used to guide therapy. These predictive factors not only aid in prognosis, but also predict which patients will benefit from therapy. These are the clinically useful prognostic factors. Examples in breast cancer include the estrogen and progesterone receptors (predict which patients will respond to hormonal therapy) and the HER2 receptor (predicts which patients will respond to Herceptin). To improve net outcomes, the 70-gene prognostic signature should not only be an independent risk factor for prognosis, but should also predict which patients will benefit from chemotherapy and which will not.

The primary outcomes of interest should be overall survival (OS), disease metastasis free survival (DMFS), and breast cancer specific survival (BCSS) over a minimum of five to ten years. The clinical benefit attributed to current gene expression profiles is a reduction in the use of chemotherapy in patients who do

not benefit from chemotherapy. Ideally, randomized trials would directly compare the results achieved using expression arrays to guide therapy to those obtained using current standards for treatment decisions. The evidence should demonstrate that therapy based on tumor gene expression levels demonstrated equivalent long-term outcomes as current approaches to targeting adjuvant therapy, while reducing the number of patients receiving chemotherapy. In the absence of studies directly comparing these two treatment strategies, data demonstrating little benefit to chemotherapy in low risk groups, but significant benefit in high risk groups would provide weaker evidence that the test provides predictive as well as prognostic information. The study should demonstrate a statistically significant interaction between the risk score and treatment on five or ten-year breast cancer outcomes.

Prognostic value

The first study explicitly using gene expression profiling to identify a set of genes that strongly predict a poor prognosis was published in January 2002.²¹ Investigators at the NCI selected 98 patients with breast cancer, including 34 who developed distant metastases within five years, 44 who were disease free at five years, 18 who carried the BRCA1 mutation, and two who carried the BRCA2 mutation. The 78 patients who did not carry a BRCA mutation all had N0. All patients were younger than 55 years old and the tumors were less than 5 cm in diameter. The investigators isolated RNA from snap-frozen tumor tissue. Each sample was hybridized in duplicate to microarrays containing approximately 25,000 human genes. The relative RNA expression level for each tumor was compared to that of RNA from a pool of equal amounts of RNA from each patient sample. The investigators initially identified a group of 5000 genes with at least a two-fold variation in expression across the tumors. Limiting the analysis to patients without a germline BRCA mutation, the investigators first identified 231 genes among the 5000 that were significantly correlated with the development of distant metastasis (correlation coefficient >0.3 or <-0.3). Next, they ranked the genes by the magnitude of the correlation coefficient. Finally, they evaluated the ability of the genes to classify the tumors by sequentially adding groups of five genes until the model's accuracy stopped improving (70 genes). Many of the genes upregulated in the final model are functionally linked to cell cycle regulation, invasion, metastasis, angiogenesis, and signal transduction. In the derivation cohort, the model correctly classified 29 out of 34 of the patients developing metastases (sensitivity 85%) and 36 out of 44 of the patients who remained disease free (specificity 82%). Since the investigators were interested in ensuring that most patients with a poor prognosis were identified as candidates for chemotherapy, they chose a lower threshold value for classifying someone as high risk to ensure that the sensitivity of the test was greater than 90%. Using the new threshold, only three out of 34 patients with poor prognosis were misclassified

(sensitivity 91%), but 12 out of 44 patients with a good prognosis were misclassified (specificity 73%). The poor prognosis signature had an odds ratio of 28 (95% CI 7-107) for distant metastases, though this dropped to 15 (95% CI 4-56) with leave-one-out cross validation. Finally, the investigators validated the model in an additional 19 young patients with N0. The model correctly classified 11 out of 12 patients who developed distant metastases (sensitivity 92%) and six out of seven patients who remained disease free for five years (specificity 86%)

Given the large number of potential gene predictors assessed (25,000), the small number of outcomes (34) and the post-hoc shift in the threshold used to define a poor prognosis, the model had strong potential for overfitting. One group has demonstrated that the 70 genes identified by the methods described by van't Veer et al. were highly dependent on the set of patients used to derive the model.⁴⁸ Many other sets of 70 genes can be found using the same data and methods that do not overlap with the 70 genes in the prognostic signature, but predict outcome equally well in the derivation and validation sets. The fact that the results of the small validation study were better than those obtained in the derivation cohort was surprising.

A second study by the same group validated the 70-gene prognosis profile in a series of 295 patients with stage I or II breast cancer who were younger than 53 years.³⁵ Approximately half of the patients had lymph node negative disease (51%). All patients were followed annually for at least five years. The median follow-up was 6.7 years. RNA expression levels were assessed using the same methods as in the prior study.²¹ The ten year survival was 54.6% among the 180 patients with a poor prognosis profile, and 94.5% among the 115 patients with a good prognosis profile. In this study, the poor prognosis signature had a hazard ratio (HR) of 5.1 (95% CI 2.9-9.0) for distant metastases. In a multivariable analysis adjusting for age, lymph node status, tumor size, tumor grade, vascular invasion, ER expression, and types of treatment, the poor prognosis signature remained strongly associated with the occurrence of distant metastases (HR 4.6, 95% CI 2.3-9.2). The prognosis profile predicted being metastasis-free and overall survival at both five and ten years in this study. The absolute differences in mortality are large enough to be clinically significant. For example, the ten year overall survival was 94.5% in the good prognosis group and 54.6% in the poor prognosis group. Similarly, in patients with lymph node negative disease, the proportion of patients free from distant metastases was 93.4% in the good prognosis group and 56.2% in the poor prognosis group.

The investigators introduced a significant methodologic flaw by including 61 of the 78 patients used to develop the 70-gene prognosis profile in this validation study. The investigators addressed this by performing subgroup analyses of previously excluded patients from the earlier study – these results were



similar to those reported for the larger set of patients. An additional confusing decision described by the investigators was the use of different thresholds to define poor prognosis (0.55 for the 61 patients from the prior study and 0.40 for all other patients). Finally, the analysis was complicated by the fact that 130 of the 295 patients received adjuvant therapy (90 chemotherapy, 20 hormonal therapy, 20 both therapies) in a non-randomized fashion. The appropriate patient population whose outcomes would be improved through use of the prognosis profile remains to be defined.

Buyse et al performed the first truly independent validation of the 70-gene prognostic signature in its commercial form, MammaPrint.³³ This study formed the basis for the FDA approval of the test. The investigators studied whether the 70-gene signature had prognostic value that was independent of the best clinical risk classifications. They selected patients from five European centers who were younger than 61 years, were diagnosed prior to 1999 with node negative breast cancers < 5 cm in diameter, and who had not been treated with adjuvant therapy. The investigators identified 403 eligible patients with frozen tumor samples. They were able to extract useable RNA from 326 of these samples (81%). An additional 19 patients were ineligible for other reasons, leaving a final validation series of 307 patients. Agendia performed the microarray analysis using MammaPrint, a custom microarray chip designed to assay the mRNA expression of the 70 genes in triplicate. The investigators defined a tumor signature as low risk if the Pearson correlation coefficient for the 70-gene profile was above 0.4. All other profile signatures were considered high risk. During a median follow-up of 13.6 years, there were 68 recurrences, 77 distant metastases and 82 deaths. The investigators used the Adjuvant! Software⁴, which uses the patient's age, tumor size and grade, ER status and nodal status, to calculate the patient's ten year probability of survival. Due to missing data, they were not able to calculate the survival probability for five patients in the validation series because of missing ER status. For dichotomous analysis, they considered ER-positive patients with estimated survival $\geq 88\%$ and ER-negative patients with estimated survival $\geq 92\%$ to be clinically at low risk of recurrence.

The ten year overall survival was 69% for patients with a high risk gene signature in both the clinical low risk and high risk groups. Similarly, the ten year survival for patients with a low risk gene signature was 88% and 89% for the clinical low risk and high risk groups respectively. The HRs of the high risk gene signature for time to distant metastases (2.32, 95% CI 1.35-4.00), overall survival (2.79, 95% CI 1.60-4.87), and disease-free survival (1.50, 95% CI 1.04-2.16) were greater than the comparable hazard ratios for age ≤ 50 , T2 tumor size, poorly differentiated tumor grade, ER-negative status, high risk by Adjuvant! Software, and high risk by the Nottingham Prognostic Index. Those considered high risk by the St.Gallen criteria had a greater



HR for disease-free survival (HR 2.18, 95% CI 0.96-4.96), but not for time to distant metastases (HR 2.22) and overall survival (HR 1.69). The sensitivity of the 70-gene signature for predicting metastases within five years was slightly higher than that of Adjuvant! (90% vs. 87%), and it had a much higher specificity (42% vs. 29%).

Of note, the initial estimates from the first validation reported from the NKI³⁵ were significantly higher than those in this validation series for time to distant metastases (HR 6.1 vs. 2.1), overall survival (HR 17.5 vs. 2.6), and disease free survival (HR 4.8 vs. 1.4). There are likely many factors that contribute to these large differences between these two “validation” studies of the 70-gene prognostic signature. There is always concern about overfitting in models developed from tens of thousands of predictors – one re-analysis suggested that this played a role in the overly optimistic estimates seen in the first validation study.²⁹ In addition, the more recent validation study of Buyse et al³³ used a different technology platform to estimate the relative expression levels of the 70 genes. They used the MammaPrint array that forms the basis of the commercially available assay and thus, is probably a more realistic estimate of how the signature will perform in the real world. Studies have demonstrated that a prediction model developed on one gene expression platform may perform with lower accuracy when evaluated on a different platform.⁴⁹ Finally, the patient populations were different: the second validation study included older women, followed them almost twice as long, and excluded patients who received adjuvant therapy.

The validation studies published since 2006³⁷⁻⁴⁶ help to address the concerns raised by Buyse et al. The characteristics of the patients in these cohorts and their outcomes are summarized in Table 1. More than 1000 additional women were studied and most using the commercial MammaPrint assay, although two of the studies included only women whose data had been published in prior studies^{42, 44} and three additional studies^{40, 45, 46} also included some women from prior studies. Many of the studies addressed the prognostic value of the 70-gene signature in specific patient subgroups including women with node positive disease⁴⁰, women with node negative disease^{37, 39}, women older than those included in the initial development and validation studies^{38, 45}, women with tumors less than 2 cm in diameter⁴⁴, and in a Japanese population.⁴¹

Table 1: Prognostic value of the 70-gene prognostic signature (MammaPrint)

Study	N	Overlap, %	FU, years	Age, years Year diagnosed Location	ER +, %	HER2+, %	LN+, %	Good prognosis, %	Chemo, %	Hormonal Tx, %	OS	DMFS	BCSS	Distant mets, %
Van't Veer 2002	78	0	2.5-8.7	<55 1983-1996 NKI	77	NR	0	45	4	3	NR	18 (3.3-94)	NR	44
Van de Vijver 2002	295	21	6.7	<52 1984-1995 NKI	77	NR	49	39	37	14	NR	4.6 (2.3-9.2)	NR	30
Buyse 2006	302	0	13.6	<61 1980-1998 5 centers	70	NR	0	37	0	NR	2.6 (1.5-4.8)	2.1 (1.2-3.8)	NR	25
Bueno-de-Mesquita 2007	427 Node negative	0	1.2	27-60 2004-2006 NKI	20%	11%	15%	51%	18%	13%	NR	NR	NR	1%
Wittner 2008	100 Post-menopausal	0	11.3	69% > 55 1985-1997 Mass General	80%	NR	0%	27%	21%	24%	NR	NR	NR	9%
Bueno-de-Mesquita 2009	123 Node negative	0	5.8	100% < 55 1996-1999 NKI	76	7	0%	52%	26	22	3.0 (1.0-8.9)	4.8 (1.3-17)		11
Mook 2009	241 with 1-3 positive LN	Unclear	7.8	100% < 71 1994-2001 NKI + Milan	79	15	100	41	53	69	*5.4 (2.1-14)	3.0 (1.0-9.0)	7.2 (1.8-28)	18
Knauer 2010	541	100	7.1		90	11	51	47	42	100		*3.9 (2.0-7.6)	*4.8 (2.0-12)	10
Kunz 2010	44	0	-	32-56 2004-2008 Germany	78	11	35	66	NR	NR	NR	NR	NR	NR
Ishitobi 2010	102 Japanese	0	7.1	<70 1998-2001 Japan	41	NR	0	20	28	73	NR	NR	NR	8
Mook 2010a	148 All age 55 - 70	Unclear	11.6	55-70 1984-1996 NKI	78	NR	0	61		18	NR	*1.8 (0.9-3.5)	1.3 (NR)	28
Mook 2010b	964 All pT1	100	7.1	NR NKI+	84	9	27	54	22	32	NR	2.4 (1.6-3.8)	3.3 (1.9-5.5)	16
Straver 2010	167 Neo-adjuvant chemotherapy	Unclear	2.1	23-68 2000-2008 NKI	> 53%	25	73	14%	100	NR	NR	NR	NR	9

* Univariate estimates – not adjusted for age, tumor size, grade, ER status, HER2 status, LN status, or use of chemotherapy or hormonal therapy.
OS overall survival; DMFS Distant metastasis free survival; BCSS Breast cancer specific survival; NKI Netherlands Cancer Institute

One of the consistent findings from these cohorts is that the 70-gene prognostic index is a much stronger risk factor for recurrence during the first five years compared to the longer follow-up. Looking at Table 1, the strength of the association for DMFS is strongest in studies with the shortest follow-up and weakest in studies with the longest follow-up. This was observed in the first validation study of Van de Vijver published in 2002: the unadjusted HR for distant metastases with a poor prognosis signature was 8.8 (95% CI 3.8 – 20) during the first five years of follow-up but only 1.8 (95% CI 0.8 – 4.5) after five years.³⁵ In Buyse et al, the HR decreased from 4.7 for the first five years to 3.5 for the first ten years and was only 2.1 (95% CI 1.2-3.8) using complete follow-up.³³ In the same study, the hazard ratios for disease free survival decreased from 2.1 at five-years to 1.7 at ten-years and 1.4 using complete follow-up. A similar pattern was observed for women 55-70 years old in the recent publication by Mook et al.⁴⁵ The adjusted hazard ratio for breast cancer specific mortality decreased from 14.4 at five-years to 2.2 at ten-years and to 1.3 overall. A different pattern was seen for the clinical high risk profile in the same study. The hazard ratio for the high risk clinical profile adjusted for the 70 gene prognostic signature mortality increased from 1.9 at five-years to 4.4 at ten-years and was 3.2 overall. These data suggest that the 70-gene prognostic signature is primarily a risk factor for metastatic disease presenting in the first five-years of follow-up and that other clinical factors are better at predicting later recurrences.

Predictive value

Knauer and colleagues published the only study evaluating the predictive value of the 70-gene prognostic signature for response to chemotherapy.⁴² They created a pooled database of patients from six prior studies including one that has yet to be published.^{35, 37, 39, 40, 45} They included women with unilateral stage T1-3, N0-1, M0 invasive breast cancer diagnosed between 1984 and 2006. A total of 541 women met those criteria and were treated with either endocrine therapy alone or chemotherapy in addition to endocrine therapy. Frozen samples from each tumor were processed using the commercial MammaPrint array at Agendia's laboratory in Amsterdam. Each tumor was classified as having a high or low risk signature: 252 (47%) as low risk and 289 (53%) as high risk. The primary endpoints were BCSS and DMFS. Multivariate models were adjusted for age at diagnosis, tumor size, number of positive lymph nodes, histologic grade, ER and PR status, hormonal therapy and chemotherapy. Median follow-up was 7.1 years, but all analyses were censored at five years. The signature was prognostic: women with a low risk tumor signature had a five-year BCSS of 97% and a five year DMFS of 95% while women with a high risk tumor signature had a five-year BCSS of 87% and a five year DMFS of 82%. However, women in both risk categories appeared to benefit from chemotherapy, although the estimates were not statistically significant in the low risk group due to lack



of power. For BCSS the unadjusted hazard ratio for chemotherapy was 0.58 (0.07-5.0) in the low risk group and 0.21 (0.07-0.59) in the high risk group. The p-value for interaction between use of chemotherapy and the risk signature was not statistically significant ($p=0.45$). For DMFS the unadjusted hazard ratio for chemotherapy was 0.26 (0.03-2.0) in the low risk group and 0.35 (0.17-0.71) in the high risk group. The p-value for the interaction was not reported, but in this case the trend was towards greater relative benefit from chemotherapy in the low risk group. Thus, these two analyses do not support a predictive role for the 70-gene prognostic score. For both outcomes, the absolute benefit from chemotherapy was greater in the high risk group as the score is prognostic: the high risk groups are at higher risk for events. For example, the five-year BCSS was 99% for the low risk patients who received chemotherapy and 97% in those who did not receive chemotherapy (absolute benefit 2%, NNT 50), while the five-year BCSS was 94% for the high risk patients who received chemotherapy and 81% in those who did not receive chemotherapy (absolute benefit 13%, NNT 8).

This study suffers from several major issues. The primary problem is that the patients were not randomized to chemotherapy and so there is undoubtedly significant selection bias. Moreover, as would be expected, it appears that few low risk patients received chemotherapy – hence the confidence intervals around the estimates of effect in the low risk groups are very wide and in the multivariable analysis, it appeared that the model was unable to converge. In addition to patient heterogeneity, there is likely significant heterogeneity in the chemotherapy as patients were diagnosed with breast cancer over more than 20 years (1984 to 2006) and the standards of care have changed considerably. In addition, censoring the follow-up at five years biased the results in favor of the utility of prognostic signature because the association between the 70-gene signature and recurrent disease falls quickly after five years of follow-up. Given that the majority of distant recurrences and deaths from breast cancer occur more than five years after diagnosis, this is a significant limitation.

Ongoing randomized trial

The Microarray In Node negative Disease may Avoid ChemoTherapy (MINDACT) trial is a randomized clinical study that will assess the value of the 70-gene prognostic signature in predicting the response to chemotherapy for patients with N0.⁵⁰⁻⁵² Women 18 to 70 years old with T1, T2, or operable T3 invasive breast cancers are eligible for enrollment. This prospective, randomized phase III study will compare risk assessment using gene expression with risk assessment using common clinical-pathological criteria (Adjuvant!)⁴ in selecting patients for adjuvant chemotherapy in node-negative breast cancer. The goal is to study 6000 women with the prognostic signature. If both the gene signature and the clinical assessment are



high risk (n=3300), patients will be randomized to one of two chemotherapy regimens. If both are low risk (n=780), then no chemotherapy will be administered. If the two forms of risk assessment are discordant (n=1920), then patients will be randomized to therapy based either on the clinical assessment or the gene expression signature. Patients with hormone receptor positive disease will be randomized to one of two hormonal regimens. As of October 14, 2009 the study had enrolled 2264 patients (http://www.eortc.be/services/unit/mindact/MINDACT_websiteii.asp#Current).

In summary, the 70-gene prognostic signature was one of the first gene expression profiles developed specifically to identify patients with a poor prognosis. There have been multiple validation studies published since the prior CTAF review in 2006 and these consistently show that the high risk signature separates early stage breast cancers into groups at high and low risk of recurrent disease, independent of usual clinical and pathologic predictors of recurrence. However, the improvements in predictive accuracy were modest and the association between the prognostic signature and recurrent disease weakens quickly after five years, while that of the traditional risk models strengthens over time. It is also unclear what subgroup of patients would derive the greatest benefit from use of the 70-gene prognostic signature. Some investigators suggest that patients with N0 and a prognostic signature that is opposite from the clinical risk assessment using current tools may benefit from therapy guided by the prognostic signature. This is being tested in the MINDACT study, a clinical trial that began recruiting patients in 2006. It is not yet clear whether the use of the prognostic signature would improve patient outcomes through increases in disease-free and overall survival or from a decrease in the number of patients unnecessarily treated with chemotherapy. Furthermore, the one study looking at the ability of the 70-gene signature to predict the response to chemotherapy failed to demonstrate a significant difference in the treatment effect by high and low risk signature subgroups. Thus, TA criterion 3 is not met for the 70-gene prognostic signature.

TA Criterion 3 is not met

TA Criterion 4: The technology must be as beneficial as any established alternatives.

There is no single established alternative to gene expression profiling for risk stratification of patients with breast cancer. Guidelines from the NCI, the NCCN, St Gallen, the Nottingham Prognostic Index, and Adjuvant! Software all rely to varying degrees on patient age, tumor size and grade, lymph node status, and hormone receptor status to risk stratify patients. The risk status is then used to guide recommendations for chemotherapy. Recommendations for chemotherapy vary significantly for early stage, N0. More recently,

the NCCN guidelines and some specialty society recommendations have included the 21-gene recurrence score (Oncotype DX) in their algorithms for deciding on chemotherapy for early stage ER+ breast cancers. No studies directly compare outcomes of chemotherapy guided by one of the guidelines noted above with chemotherapy guided by risk classification by gene expression profiling.

In the absence of prospective, randomized data one study evaluated the ability of the 70-gene prognostic score to predict response to chemotherapy⁴² and two studies evaluated similar outcomes for the 21-gene recurrence score.^{53, 54} The results of the three studies are summarized in Table 2. The results from Knauer et al were described above under TA 3. From Table 2 it is clear that there is no evidence that the 70-gene prognostic signature predicts response to chemotherapy as the relative hazards are similar in the high and low risk groups, while there is clear and statistically significant evidence that the 21-gene recurrence score predicts response to chemotherapy in both lymph node negative and lymph node positive study populations. Furthermore, the studies of the 21-gene test were nested within randomized trials, so there is unlikely to be significant selection bias and confounding, while the study of the 70-gene signature is likely to have significant bias and confounding.

Table 2: Predictive value of the 70-gene prognostic signature* (MammaPrint) compared with that of the 21-gene recurrence score (Oncotype DX)

Study	Assay	Population	Study design	HR for chemotherapy in low risk group	HR for chemotherapy in high risk group	P value for interaction
Knauer 2010	70-gene (MammaPrint)	Heterogeneous ER+, ER-, LN+, LN-	Subsets of six or seven cohorts	0.58 (0.07-5.0) BCSS 0.26 (0.03-2.0) DMFS	0.21 (0.07-0.59) BCSS 0.35 (0.02-0.71) DMFS	0.45 BCSS NR DMFS
Paik 2006	21-gene (Oncotype DX)	Homogeneous ER+ LN-	Nested Phase 3 RCT	1.31 (0.46-3.8) DMFS	0.26 (0.13-0.53) DMFS	0.038
Albain 2010	21-gene (Oncotype DX)	Homogeneous ER+ LN+	Nested Phase 3 RCT	0.95 (0.59-1.52) DFS	0.57 (0.39-0.83) DFS	0.029

* All results are unadjusted

BCSS Breast cancer specific survival; DMFS Distant metastasis free survival; DFS Disease free survival

One study directly compared risk prediction using the 70-gene prognostic score to the recurrence score and several other gene profiles.⁵⁵ The Recurrence Score was not calculated using the RT-PCR based commercial Oncotype DX assay, but was estimated using data on the 21 genes from the expression arrays



results used to develop the 70-gene prognostic score. Similarly, a special algorithm was used to approximate the Perou / Sorlie intrinsic subtype classification. The study was performed using the 295 tumor samples that contributed to the initial derivation and validation of the 70-gene profile and the wound response model.^{35, 36} The study sample included a mix of ER-positive (n=225) and ER-negative (n=70) breast cancers. Treatment was also heterogeneous: 20 received tamoxifen alone, 20 received tamoxifen plus chemotherapy, and 90 received chemotherapy alone. Even though there was very little overlap between the genes included in the four profiles based on multiple genes, there was a high degree of concordance in outcome predictions. This was particularly true for the 70-gene prognostic signature and the recurrence score. The two classifiers agreed on 81% of the samples (239/295). The authors suggest that the four classifiers based on gene expression profiling may be representing the same underlying biological phenotype. If true, they may be able to be used interchangeably. Ideally subsets of patients in the two ongoing randomized trials will be assessed with both tests to better delineate their respective strengths and weaknesses.

TA Criterion 4 is not met .

TA Criterion 5: The improvement must be attainable outside the investigational setting.

Several studies have been published on the reproducibility of the 70-gene prognostic signature.^{56, 57} The multi-center validation study suggests that it is possible to attain useful data from specimens obtained at multiple sites, although part of the explanation for the weaker association between the prognostic signature and breast cancer recurrence in that study may be variability in specimen handling and measurement error.

Since the topic was last reviewed, standard protocols and new reagents have been specifically designed to preserve mRNA for gene expression profiling. Reduction in the noise introduced by RNA degradation during sample processing, storage, and preparation should increase the precision of the measurements of mRNA levels and increase the power to predict important clinical outcomes using gene expression profiling. However, problems with reagents can have important effects on test results. This has recently been an issue for MammaPrint:

“April 14, 2010: Faulty Reagent Caused 'Marginal' Over-Reporting of Breast Cancer Recurrence With MammaPrint. An Agendia official said that the company has provided "new, corrected MammaPrint reports"



for all of the physicians who received faulty results and has also implemented internal quality-control measures to guard against a similar issue in the future.” - GenomeWeb Pharmacogenomics Reporter

“May 14, 2010: FDA Provides Notice on Agendia's Ongoing MammaPrint Recall: The FDA's April 21 notice said that over a period of about six months, approximately 15 percent of MammaPrint results over-reported the chance of metastasis risk as 29 percent risk of recurrence instead of 10 percent.” - GenomeWeb Pharmacogenomics Reporter

Furthermore, TA criterion 5 cannot be met if TA criteria 3 and 4 are not met.

TA Criterion 5 is not met

CONCLUSION

The majority of breast cancers in the United States are diagnosed at an early stage. Significant improvements in long-term outcomes for women with breast cancer have been achieved by targeting therapy based on the results of tests that predict response to therapy (hormone receptor status for tamoxifen and aromatase inhibitors; HER2 status for trastuzumab). Many women with early stage tumors receive no benefit from chemotherapy, but suffer all of its side effects. Current guidelines and risk assessment tools recommend that 80 to 90% of these women be offered chemotherapy, but fewer than 50% will benefit. The primary clinical goal of the current gene expression profile tools is to improve risk stratification of women with early stage breast cancers in order to more precisely individualize use of chemotherapy.

Gene expression profiling describes several technologies that quantify the relative expression of mRNA levels for many genes. Patterns of gene expression can be used to differentiate one tumor type from another and to separate tumors likely to be associated with a good prognosis from those with a poor prognosis. The MammaPrint test is the commercial version of the 70-gene prognostic signature developed at the NKI in Amsterdam. It was designed to predict five year rates of distant metastases in younger women with N0. The interpretation of the results from the initial validation study in 295 patients was clouded by the inclusion of patients used to develop the signature in the group of patients used to validate the signature, the inclusion of a large number of women with lymph node positive breast cancer (49%), and the use of a different array platform than the commercial test. A larger validation study of 307 patients with lymph node



negative disease < 5 cm in diameter was the first to use the commercial microarray. Multiple retrospective cohorts have demonstrated that the 70-gene prognostic signature performed better than standard guidelines and risk assessment tools at predicting recurrent disease, but the magnitude of the association with the poor prognostic signature decreases with length of follow-up. Recent studies have confirmed that the 70-gene prognostic signature adds prognostic information in older women and in women with lymph node negative or up to three positive lymph nodes. However, it remains unclear if the 70-gene prognostic signature can predict response to chemotherapy. One study pooled retrospective data from multiple cohorts to evaluate this question. The study was underpowered to evaluate the response to chemotherapy in the low risk group and it was based on observational data rather than randomized data, so the results may not be accurate. That study reported that chemotherapy produced non-significant, but potentially clinically important 50% to 70% reductions in breast cancer recurrence in the low risk group that were approximately equivalent to the reductions observed in the high risk group. These data are in contrast with that for the 21-gene recurrence score which has two randomized trials demonstrating a statistically significant interaction between the recurrence score and chemotherapy in predicting response to chemotherapy. In both trials, women with low recurrence scores who were randomized to chemotherapy had similar outcomes to those randomized to no chemotherapy. Women with high recurrence scores who were randomized to chemotherapy had much better outcomes than similar women randomized to no-chemotherapy. In summary, a large number of patients have been evaluated with the commercial version of the 70-gene prognostic signature and the test clearly offers prognostic information beyond that offered by standard tools. However it remains unclear how the 70-gene prognostic signature should be used in managing patients with early stage breast cancer. A large, randomized clinical trial (MINDACT) enrolling 6000 patients in Europe will test the hypothesis that use of the 70-gene prognostic score can improve outcomes for women with early stage breast cancer.

DRAFT RECOMMENDATION

It is recommended that the use of the 70-gene prognostic signature (MammaPrint) does not meet Technology Assessment Criterion 3 through 5 for safety, effectiveness and improvement in health outcomes.

June 2, 2010

This is the second assessment of this technology by CTAF.



RECOMMENDATIONS OF OTHERS

Blue Cross Blue Shield Association (BCBSA)

A November 2007 BCBSA Technology Evaluation Center review of Gene Expression Profiling of Breast Cancer to Select Women for Adjuvant Chemotherapy found in part that:

The use of MammaPrint® or the Breast Cancer Gene Expression Ratio to determine recurrence risk in women with early stage breast cancer does not meet the TEC criteria.

Centers for Medicare and Medicaid Services (CMS)

The local Medicare carrier for California has developed a coverage policy for the use of MammaPrint effective November 2009.

American College of Obstetricians and Gynecologists (ACOG)

ACOG has been invited to provide an opinion and to have a representative attend the meeting.

American Society of Breast Surgeons (ASBS)

The ASBS has been invited to provide an opinion and to have a representative attend the meeting.

Association of Northern California Oncologists (ANCO)

ANCO has provided an opinion regarding this technology. A representative will not be attending the meeting.

Medical Oncology Association of Southern California (MOASC)

MOASC has been invited to provide an opinion on the use of this technology and representation at the meeting.

American Cancer Society (ACS)

ACS has been invited to provide an opinion and to have a representative attend the meeting.

ABBREVIATIONS USED IN THIS ASSESSMENT:

CTAF	California Technology Assessment Forum
NCCN	National Comprehensive Cancer Network
TNM	Tumor node metastasis
T	Tumor
N	Axillary lymph nodes
M	Distant metastasis
HER2	Human Epidermal Growth Factor Receptor
ER	Estrogen receptor
NCI	National Cancer Institute
mRNA	Messenger RNA
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
RT-PCR	Reverse-transcriptase polymerase chain reaction
cDNA	Complementary DNA
NKI	Netherlands Cancer Institute
N0	Lymph node negative breast cancers
FDA	US Food and Drug Administration
IVDMIA	In Vitro Diagnostic Multivariate Assays
PMA	Pre-market approval
DARE	Database of Abstracts of Reviews of Effects
OS	Overall survival
DMFS	Disease metastasis free survival
BCSS	Breast cancer specific survival
HR	Hazard ratio
CI	Confidence interval
MINDACT	Microarray In Node negative Disease may Avoid ChemoTherapy

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