

Gene Expression Profiling of Breast Cancer to Select Women for Adjuvant Chemotherapy



Assessment
Program
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Executive Summary

Background

For women with early stage breast cancer, adjuvant chemotherapy provides a significant proportional benefit, i.e., benefit that is the same regardless of prognosis, and regardless of hormonal treatment for hormone-receptor-positive tumors. However, in all cases, the absolute benefit of chemotherapy depends on the baseline risk of recurrence. For example, women with the best prognosis have small tumors that are estrogen-receptor (ER) positive, and disease that is lymph-node negative. After tamoxifen therapy, these women have an approximately 15% baseline risk of recurrence. On average, this population also receives a small, but significant, absolute benefit from chemotherapy. However, approximately 85% of these patients would be disease free at 10 years with tamoxifen treatment alone. Current risk classifiers do not accurately identify those early stage patients who are at low risk of recurrence; as a result, more patients are treated with chemotherapy than can benefit. Better predictors of baseline risk could help women who prefer to avoid the toxicity of chemotherapy, if assured that their risk is low, make better treatment decisions in consultation with their physicians.

Conventional risk classifiers include the National Comprehensive Cancer Network guidelines, St. Gallen consensus recommendations, and Adjuvant! Online. These classifiers estimate recurrence risk by considering criteria such as tumor size, type, grade, and histologic characteristics; hormone receptor status; and lymph node status. Clinical trial data and physician experience support the development and regular updates of these classifiers, and studies show significant predictive ability. However, no single classifier is considered a gold standard, and several common criteria have qualitative or subjective components that add variability to risk estimates. Moreover, differences among criteria or their use in different classifiers may result in significantly different risk estimates for the same patients. Thus, quantitative risk predictors with greater accuracy are needed.

Recently, several groups have identified panels of gene expression markers (“signatures”) that appear to predict the baseline risk of breast cancer recurrence after surgery, radiation therapy, and hormonal therapy (for ER-positive tumors) in women with node-negative disease. If these panels are more accurate than current conventional classifiers, they could be used to aid chemotherapy decision-making, in cases where current guidelines only advise considering chemotherapy without strong recommendations either way, without negatively affecting disease-free and overall survival outcomes.

Three gene expression tests for evaluating recurrence risk in early stage breast cancer are commercially available in the U.S. at the time of this writing. Genomic Health, Inc. (Redwood City, CA) developed and markets Oncotype DX™. AviraDx, Inc. (Carlsbad, CA) licensed the Breast Cancer Gene Expression Ratio to Quest Diagnostics (test also known as the 2-gene ratio or HOXB13/IL-17BR ratio) and also offers the test at its in-house laboratory facility. MammaPrint® was commercially developed by Agendia (the Netherlands; test originally referred to as the 70-gene signature) and is

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the only test that is cleared by the U.S. Food and Drug Administration (FDA). However, MammaPrint® is performed only in the Agendia Netherlands laboratory, and U.S. samples must be sent there using a sample collection kit provided by the laboratory. This Assessment will focus on all three tests. Other multigene test panels are in various stages of development and will not be evaluated here.

Objective

This Assessment examines whether, compared to conventional risk assessment tools, the use of gene expression profiling improves outcomes when used to decide whether risk of recurrence is low enough to forego adjuvant chemotherapy for early stage breast cancer.

Search Strategy

For this update, MEDLINE® was searched (via PubMed) for “Breast Neoplasms”[MeSH] AND (“Gene Expression Profiling”[MeSH] OR “Gene Expression”[MeSH]) from January 2005 through December 2007.

Selection Criteria

Included studies were full-length journal publications reporting on the use of gene expression panels to predict breast cancer recurrence for the purpose of identifying women with early stage breast cancer who were likely to benefit from postoperative adjuvant chemotherapy. Unpublished studies that analyzed data from published studies, where the full presentation was available, were also accepted as evidence.

We included studies reporting evidence of statistical association between gene expression profiling results and patient outcomes (disease recurrence), which comprised the majority of the evidence. However, while associational evidence is a necessary first step and is useful for making predictions regarding populations, it is not sufficient evidence of improved risk prediction for individual patients. Therefore, in the absence of prospective clinical trials of clinical utility, we also searched for studies reporting either reclassification studies (individual patient risk first classified by conventional clinical criteria, then reclassified by gene expression profile result), or receiver operating curve (ROC) analysis.

Main Results

Oncotype DX™ The Oncotype DX™ gene expression profile was validated in studies that used archived tumor samples (obtained at surgery) where available from subsets of patients enrolled in already completed, randomized, controlled trials of treatment for ER-positive, node-negative breast cancer. Results from the Oncotype DX™ gene panel are combined into a recurrence score (RS).

Retrospective epidemiologic analyses of these samples indicate strong, independent associations between Oncotype DX™ RS results and distant disease recurrence or death from breast cancer (Table; Paik et al. 2004a, Habel, 2006). In additional studies using the data from Paik 2004a (Paik et al. 2004b; Bryant 2005), patient risk levels were individually classified by conventional risk classifiers, then reclassified by Oncotype DX™.

Results indicate that Oncotype DX™ adds additional risk information to the conventional clinical classification of individual high-risk patients, and identifies a subset of patients who would otherwise be recommended for chemotherapy, but are actually at lower risk of recurrence (average 7–9% risk at 10 years; upper 95% confidence interval [CI] limits, 11–14%). Oncotype DX™ testing also identifies a subset of conventionally classified low-risk patients who are reclassified at higher risk of recurrence. However, due to wide confidence intervals, it is not clear that all reclassified higher-risk individuals would realize a net benefit from chemotherapy.

A community-based study of women with ER-positive tumors provides supportive evidence of clinical validity with results of a strong association between RS results and risk of death from breast cancer. Finally, a study in which samples from a randomized, controlled trial of ER-positive, node-negative breast cancer patients treated with tamoxifen versus tamoxifen plus chemotherapy were tested by Oncotype DX™ showed that RS high-risk patients derived clear benefit from

Table. Summary of Oncotype DX™ RS and Recurrence Risk Studies

Study	Study Type	Total n	Study Objective	Results			
Paik et al. 2004a	TAM arm of NSABP B-14 RCT	668	Predict recurrence	RS Risk	% of patients	K-M Distant recurrence at 10 yr, % (95% CI)	
				Low (<18)	51	6.8 (4.0–9.6)	
				Int (18–30)	22	14.3 (8.3–20.3)	
				High (>31)	27	30.5 (23.6–37.4)	
All	100	15 (12.5–17.9)					
Paik et al. 2004b	Additional analysis of Paik et al. 2004a data	668	Reclassification study; determine incremental risk compared to conventional classifier	Risk Classification by NCCN¹	Risk Reclassification by Oncotype DX	n	% DRF at 10 yr, (95% CI)²
				Low (8%)	Low	38	100 (NR)
					Int	12	80 (59–100)
					High	3	56 (13–100)
				High (92%)	Low	301	93 (89–96)
					Int	137	86 (80–92)
	High	178	70 (62–77)				
Bryant 2005	Additional analysis of Paik et al. 2004a data	668	Reclassification study; determine incremental risk compared to conventional classifier	Risk Classification by Adjuvant! Online¹	Risk Reclassification by Oncotype DX	n	% recurrence at 10 yr (95%CI)²
				Low (53%)	Low	216	5.6 (2.5–9)
					Int-High	138	12.9 (7–19)
				Int-High (47%)	Low	122	8.9 (4–14)
	Int-High	192	30.7 (24–38)				
Habel et al. 2006	Case-control	255 ER+ TAM+; 361 ER+ TAM-	Predict mortality	RS Risk	10-yr Absolute Risk of Death, % (95% CI) ER+, TAM-treated	10-yr Absolute Risk of Death, % (95% CI) ER+, No TAM	
				Low (<18)	2.8 (1.7–3.9)	6.2 (4.5–7.9)	
				Int (18–30)	10.7 (6.3–14.9)	17.8 (11.8–23.3)	
				High (≥31)	15.5 (7.6–22.8)	19.9 (14.2–25.2)	

Abbreviations

DRF: distant recurrence free; ER: estrogen receptor; int: intermediate; K-M: Kaplan Meier; N: total number of patients; NCCN: National Comprehensive Cancer Network; NR: not reported; RS: Oncotype DX™ recurrence score; NSABP, National Surgical Adjuvant Breast and Bowel Project; RCT: randomized controlled trial; TAM: tamoxifen

¹ Percentages are percent of total N.

² Estimated from graphs. Note that different outcomes were reported between Paik et al. 2004b and Bryant 2005 and could not be converted to similar outcomes with confidence intervals.

References

Bryant J. (2005). Toward a more rational selection of tailored adjuvant therapy data from the National Surgical Adjuvant Breast and Bowel Project. 2005 St. Gallen Breast Cancer Symposium. [Complete slide presentation via Genomic Health]

Habel LA, Quesenberry CP, Jacobs MK et al. (2006). A population-based study of tumor gene expression and risk of breast cancer death among lymph node-negative patients. *Breast Cancer Res*, 8(3):R25. Epub 2006 May 31.

Paik S, Shak S, Tang G et al. (2004a). A multi-gene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*, 351:2817-26.

Paik S, Shak S, Tang G et al. (2004b). Risk classification of breast cancer patients by the Recurrence Score assay: comparison to guidelines based on patient age, tumor size, and tumor grade. *Breast Cancer Res Treat*, 88(Suppl 1):A104 [Abstract].

chemotherapy, whereas, the average benefit for other patients was not statistically significant, although the confidence intervals were wide and included the possibility of a small benefit.

MammaPrint®. Validation studies of the MammaPrint® gene signature were conducted using banked samples from consecutive series or other convenience samples, rather than samples from randomized, controlled clinical trials. Patients and tumor samples had variable clinical characteristics, making it difficult to characterize the patients who may benefit from this test. Adjusted hazard ratios for distant metastases suggest that the test provides recurrence risk information in addition to conventional classification criteria; the strongest associations appear in the first 5 years of follow-up. Average 10-year disease-free recurrence in low-risk patients by MammaPrint® was approximately 85–88% in two studies, with a lower confidence limit of 74–79%. However, ROC analysis in an independent multicenter validation study suggests only slightly improved predictive accuracy for time to distant metastases with MammaPrint® compared to other conventional criteria. In one study, after Adjuvant! Online risk classification, patients reclassified as low risk by the 70-gene signature in either Adjuvant! Online risk group had 10-year disease-free survival rates of 88–89%, with lower confidence limits of 74–77%. Patients reclassified as high risk had 10-year disease-free survival rates of 69%, with lower confidence limits of 45–61% and upper confidence limits of 76–84%.

Breast Cancer Gene Expression Ratio. The Breast Cancer Gene Expression ratio was significantly and independently associated with poorer disease-free survival in two studies of lymph-node-negative, ER-positive, tamoxifen-treated patients with breast cancer. Patients who were low risk by the 2-gene expression ratio had average 10-year recurrence rates of about 17–25%. Two additional studies in heterogeneous populations of patients also support a statistical association between the 2-gene expression ratio and recurrence-free survival. No ROC or reclassification analyses show whether the Breast Cancer Gene Expression Ratio better classifies conventionally classified high-risk patients according to recurrence outcomes.

Author’s Conclusions and Comments

Oncotype DX™ 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, estrogen-receptor positive breast cancer who were treated with tamoxifen and who met other specific trial enrollment criteria. Additionally, Oncotype DX™ provides information about the risk of recurrence that is incremental to conventional classifiers used to predict risk. Women classified as high risk by conventional methods and reclassified as low risk by Oncotype DX™ have a recurrence of at most 10–14% and likely less at lower RS values in the low-risk spectrum.

Chemotherapy provides the same proportional benefit to all patients, but the absolute benefit will be very low when the prior risk is low; such a low absolute benefit may not be perceived by patient and physician as outweighing the harms of chemotherapy. Thus, a woman who prefers to avoid the toxicity and inconvenience of chemotherapy and whose Oncotype DX™ RS value shows that she is at very low risk of recurrence might reasonably decline chemotherapy. The lower the RS value, the greater the confidence the woman can have that chemotherapy will not provide net benefit; outcomes are improved by avoiding chemotherapy toxicity.

Recently, several organizations have updated their guidelines for recommended therapy at different levels of risk determined by Oncotype DX™:

- The American Society of Clinical Oncology (ASCO) “2007 Update of Recommendations for the Use of Tumor Markers in Breast Cancer” indicates that the Oncotype DX™ assay can be used to predict the risk of recurrence in newly diagnosed patients with node-negative, estrogen-receptor positive breast cancer, who will be treated with tamoxifen; and to identify patients who may not require adjuvant chemotherapy. The recommendations state, “Although performed retrospectively, the validation of this assay using a prospectively collected clinical trial data set, but retrospectively collected tissues from the data set, might be considered as Level of Evidence I [best] for use of this assay.”

- The National Comprehensive Cancer Network (NCCN) Breast Cancer Clinical Practice Guidelines (2008) indicate that the “21-gene RT-PCR assay” (Oncotype DX™) can be considered for patients with hormone receptor-positive, HER2-negative disease and tumor greater than 1 cm in size, or 0.6–1 cm and moderately/poorly differentiated or with unfavorable features, prior to making decisions about optional chemotherapy. The recommendation is categorized as “nonuniform NCCN consensus (but no major disagreement), based on lower level evidence and clinical experience.”
- The 10th St. Gallen (Switzerland) expert consensus meeting summary stated that “the Panel did not accept the molecularly based tools such as Oncotype DX™ ...as sufficiently established to define risk categories.”

Thus, there is limited agreement that some women may benefit from using the results of the test to guide chemotherapy decisions. However, there are several limitations to the available evidence:

- Among those willing to be guided by the test result, it is unknown what proportion of conventionally estimated intermediate- to high-risk patients will have sufficiently low RS values to change their decision regarding chemotherapy.
- The recurrence risk level below which women are comfortable without chemotherapy is unknown; how the presentation of risk information affects choices is also unknown.
- Women reclassified by RS result as intermediate or high risk from conventionally estimated low risk have a much wider range of recurrence risk estimates; women with very high RS values are likely to benefit from accepting chemotherapy, but for the intermediate-risk group benefits are uncertain.
- Because the RS is a continuous function with respect to recurrence rates, risk category cutoff values selected by the test developer are arbitrary and may not be optimal.

In addition, some patient evaluation and treatment regimens in the validation studies differ from current practices. Patients in these studies were women younger than 70 years of age (or with a life expectancy of at least 10 years) who had unilateral, non-fixed, ER-positive, node-negative (by full axillary dissection) carcinomas and who were treated with surgery (mastectomy or lumpectomy), radiation therapy, and tamoxifen. In one trial, patients in the experimental arm were also treated with CMF (cyclophosphamide, methotrexate, and 5-fluorouracil) chemotherapy. Current practices differ with respect to hormonal therapies, chemotherapy regimens, techniques for assessing lymph node status, and understanding of the role of progesterone receptor (PR) positivity.

Differences in current practices compared to the validation studies do not change our findings with respect to utility of gene expression profiling using Oncotype DX™. Aromatase inhibitor (AI)-based hormonal therapy instead of tamoxifen therapy would likely reduce recurrence rates for all RS risk groups. Thus, if a patient declined chemotherapy today on the basis of a low-risk RS (risk categories defined by outcomes after tamoxifen treatment), the even lower risk associated with AI treatment would not change that decision. For those receiving anthracycline-based chemotherapy instead of CMF, the type of chemotherapy does not change the interpretation of the Oncotype DX™ risk estimate. While current practice largely involves a detailed histologic examination of sentinel lymph nodes allowing for the detection of micrometastases (less than 2 mm in size), lymph nodes with micrometastases are not considered positive for purposes of treatment recommendations. Finally, only women with ER-positive tumors were enrolled in Oncotype DX™ validation studies, whereas current clinical guidelines include either ER or PR positivity in the treatment pathway for women with hormone-receptor-positive early breast cancer. Recent studies show that ER-negative, PR-positive patients also tend to benefit from hormonal therapy.

Many of these limitations and differences in treatment will be addressed by the TAILORx trial, currently underway (total accrual goal ~10,000). This trial will determine disease recurrence outcomes of RS intermediate patients (RS 11–25, ~44%) in particular by randomizing ER- and/or PR-positive, lymph-node-negative (by current methods) patients with intermediate-risk RS results to hormonal therapy (either tamoxifen or AI-based) plus chemotherapy versus hormonal therapy alone. Low-risk patients (RS<11, ~29%; treated only with hormonal therapy) will also be followed

and compared to a prespecified target of no more than 5% recurrence at 10 years. The high-risk group (RS>25, ~27%) is assumed to benefit from chemotherapy. However, the TAILORx protocol uses more conservative cutoff values to define low- and high-risk patients than used in assay validation studies, in order to help define optimal cutoff values.

The TAILORx trial is not enrolling hormone-receptor-positive, early breast cancer patients whose disease is also HER2 positive. For these patients, the current NCCN guidelines recommend a different treatment pathway once the tumor measures 1 cm or more. Published trials of trastuzumab therapy have all included concurrent chemotherapy treatment and enrolled patients with a minimum tumor size of 1 cm or more. For these patients, trial outcomes have resulted in recommendations for trastuzumab and chemotherapy in addition to hormonal therapy. Therefore, because a choice regarding chemotherapy is not indicated, these patients are not candidates for Oncotype DX™. However, as noted in the summary of the most recent St. Gallen consensus conference and resulting guideline, “The role of trastuzumab in patients with small, endocrine responsive tumors and no axillary node involvement has not been adequately evaluated.” Thus, patients with hormone-receptor- and HER2-positive tumors that are smaller than 1 cm may need to decide whether to undergo chemotherapy alone and might be considered candidates for Oncotype DX™. It should be noted, however, that HER2 is represented in the Oncotype DX panel and RS results for the limited number of HER2-positive patients in one study were all categorized as intermediate or high risk.

MammaPrint®. There is insufficient evidence to determine whether MammaPrint® is better than conventional risk assessment tools in predicting recurrence. The 10-year disease-free survival rate of patients classified as low risk was 88-89%, with lower confidence limits of 74–77%, likely too low for most patients and physicians to consider forgoing chemotherapy. One reclassification study suggests that MammaPrint® adds additional information to one conventional risk classifier; ROC analysis suggests only a small improvement with MammaPrint® classification compared to a conventional classifier. Neither ASCO, NCCN, nor St. Gallen guidelines recommend the use of MammaPrint®. A prospective, randomized trial (MINDACT) is underway to evaluate outcomes of using MammaPrint® to guide treatment.

Breast Cancer Gene Expression Ratio. There is insufficient evidence to determine whether the Breast Cancer Gene Expression Ratio is better than conventional risk assessment tools in predicting recurrence. Recurrence rates of patients classified as low risk in available studies were 17–25%, likely too high for most patients and physicians to consider forgoing chemotherapy. There are no reclassification studies to directly compare the Breast Cancer Gene Expression Ratio with conventional risk classifiers. Neither ASCO, NCCN, nor St. Gallen guidelines recommend the use of the Breast Cancer Gene Expression Ratio.

Based on the available evidence, the Blue Cross and Blue Shield Medical Advisory Panel made the following judgments about whether gene expression profiling for managing breast cancer treatment meets the Blue Cross and Blue Shield Association Technology Evaluation Center (TEC) criteria.

1. The technology must have final approval from the appropriate governmental regulatory bodies.

Of the three gene expression profiles commercially available in the U.S., only MammaPrint® has been cleared by the FDA (cleared February 6, 2007). MammaPrint is the first cleared “in vitro diagnostic multivariate index assay” (IVDMIA). On September 7, 2006, the FDA issued new draft guidance for IVDMIA; subsequent to extensive public commentary, a second draft guidance was issued on July 26, 2007 (<http://www.fda.gov/cdrh/oivd/guidance/1610.pdf>). In the latter document, an IVDMIA is defined as one that “1) Combines the values of multiple variables using an interpretation function to yield a single, patient-specific result (e.g., a “classification,” “score,” “index,” etc.), that is intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment or prevention of disease, and 2) Provides a result whose derivation is non-trans-

parent and cannot be independently derived or verified by the end user.” The comment period for the second IVDMA draft guidance has ended, but the final disposition of the guidance is unknown at the time of this writing.

Oncotype DX™ and the Breast Cancer Gene Expression Ratio are each available from only one laboratory and are not cleared by the FDA. Clinical laboratories may develop and validate tests in-house (laboratory-developed tests or LDTs; previously called “home-brew”) and market them as a laboratory service; LDTs must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Laboratories offering the service must be licensed by CLIA for high-complexity testing. While the FDA has technical authority to regulate LDTs, to date, there has been no active oversight, with the new exception of IVDMA devices.

Gene expression tests that are currently being marketed for clinical use or are being used in research protocols classify patients into disease risk or treatment response categories using algorithms that incorporate the tumor expression status of multiple genes. As such, most or all of these assays are likely to fall into the IVDMA category. Thus, it is expected that Oncotype DX™ and possibly the Breast Cancer Gene Expression Ratio would need to meet the final pre- and postmarketing device requirements once the guidance document is finalized.

In general, FDA review of laboratory tests focuses largely on technical performance, assuring the reliability of test results over time. Review of clinical performance is more limited and may be based on “existing clinical data, new clinical trial data, review of information in the literature, or current clinical knowledge” (FDA Office of In Vitro Diagnostics 510(k) Workshop, April 19-20, 2005).

2. The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes.

Oncotype DX™ The evidence is sufficient to permit conclusions regarding health outcomes. Technical performance of the assay is well documented and is unlikely to be a major source of variability; rather, tissue sampling is likely the greatest source of variability. Retrospective epidemiologic analyses indicate strong, independent associations between Oncotype DX™ RS result and distant disease recurrence or death from breast cancer. The evidence identifies a subset of conventionally classified, high-risk patients who are at sufficiently low risk of recurrence by Oncotype DX™ that they might reasonably decide that the harms (toxicity) of chemotherapy outweigh the very small absolute benefit. Two studies of the original validation data, in which conventionally classified patients were reclassified by Oncotype DX™ result, indicate that the test provides significant recurrence risk information in addition to conventional criteria for individual patient risk classification. Additional evidence indicates that Oncotype DX™ results are significantly associated with breast cancer death in a community-based patient population, and that RS high-risk patients clearly benefit from chemotherapy, whereas benefits for other RS categories are not statistically significant.

MammaPrint®. There is insufficient evidence to determine whether MammaPrint® is better than conventional risk assessment tools in predicting recurrence. Limited technical performance evaluation of the commercial version of the assay suggests good reproducibility. The 10-year disease-free survival rate of patients classified as low risk was 88–89%, with lower confidence limits of 74–77%, likely too high for most patients and physicians to consider forgoing chemotherapy. One reclassification study suggests that MammaPrint® adds additional information to one conventional risk classifier; ROC analysis suggests only a small improvement with MammaPrint® classification compared to a conventional classifier.

Breast Cancer Gene Expression Ratio. There is insufficient evidence to determine whether the Breast Cancer Gene Expression Ratio is better than conventional risk assessment tools in predicting recurrence. Assay configuration and performance characteristics of the commercially available version of the test have not been published. Recurrence rates of patients classified as low

risk in available studies were 17–25%, likely too high for most patients and physicians to consider forgoing chemotherapy. There are no reclassification studies to directly compare the Breast Cancer Gene Expression Ratio with conventional risk classifiers.

3. The technology must improve the net health outcome; and

4. The technology must be as beneficial as any established alternatives.

Oncotype DX.™ Oncotype DX™ gene expression profiling can improve net health outcome in women with unilateral, non-fixed, hormone receptor-positive, node-negative breast cancer. In a significant subset of cases, Oncotype DX™ is likely to change the therapy decisions a patient and her physician would otherwise make using conventional risk classifiers. Women whose Oncotype DX™ RS value shows that they are at very low risk of recurrence might reasonably choose to forgo the harms and inconvenience of chemotherapy. The lower the RS value, the greater the confidence that the woman can have that chemotherapy will not provide net benefit, thus improving outcomes. Several limitations to the available evidence indicate the need for additional study.

MammaPrint® and Breast Cancer Gene Expression Ratio. The evidence is insufficient to permit conclusions as to whether the use of MammaPrint® or the Breast Cancer Gene Expression Ratio to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy improves net health outcomes in women with early stage breast cancer.

5. The improvement must be attainable outside the investigational settings.

For Oncotype DX™, there is a single-source laboratory conducting the test; this laboratory also performed the tests for the validation studies. It is expected that the quality of diagnostic performance obtained in practice should be similar to that obtained in the published studies; however, the effect of increased demand for the test on the capacity of a single-source laboratory is unknown.

Whether MammaPrint® or the Breast Cancer Gene Expression Ratio improves the net health outcome has not been established in the investigational setting.

Based on the above, the use of Oncotype DX™ to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy in women with unilateral, non-fixed, hormone receptor-positive, node-negative breast cancer who will receive hormonal therapy meets the TEC criteria. 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.

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Assessment Objective

For women with early stage breast cancer, adjuvant chemotherapy provides a significant proportional benefit, i.e., benefit that is the same regardless of prognosis, and regardless of hormonal treatment for hormone-receptor-positive tumors. However, a large proportion of these women would be disease-free at 10 years without systemic therapy or with hormonal treatment alone; only a small proportion actually derive benefit from chemotherapy. Current risk classifiers do not accurately identify those early stage patients who are at low risk of recurrence; as a result, more patients are treated with chemotherapy than can benefit. Better predictors of baseline risk could help women who prefer to avoid the toxicity of chemotherapy, if assured that their risk is low, make better treatment decisions in consultation with their physicians.

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panels are in various stages of development and will not be evaluated here.

This Assessment examines whether, compared to conventional risk assessment tools, the use of gene expression profiling improves outcomes when used to decide whether risk of recurrence is low enough to forego adjuvant chemotherapy for early stage breast cancer.

Background

Postoperative Adjuvant Therapy in Early Stage Breast Cancer

Treatment with 5 years of tamoxifen confers substantial proportional and absolute reductions in disease recurrence and death due to breast cancer in women with ER-positive breast cancer, largely independent of age, chemotherapy treatment, or other tumor characteristics (Early Breast Cancer Trialists’ Collaborative Group [EBCTCG] 2005; Carlson et al. 2006). Such benefits are not seen in women with ER-negative tumors, who have a poorer baseline prognosis. Thus, tamoxifen treatment became a standard for ER-positive tumors. Currently, in postmenopausal women, incremental benefits of aromatase inhibitors (AI) compared to tamoxifen are driving a preference for AI-based treatment (Lin and Winer 2008).

Women with tumors that overexpress HER2 have poorer recurrence and survival outcomes than women with HER2-negative disease without specific treatment. HER2 is considered a weak to moderate negative prognostic factor (Yamauchi et al. 2001), and also a strong predictor of response to specific treatment. Women with HER2-positive tumors larger than 1 or 2 cm show large proportional and absolute disease recurrence reductions across all subgroups when treated with trastuzumab (Romond et al. 2005; Piccart-Gebhart et al. 2005). Women without HER2-overexpressing tumors show no measurable benefit. Recent studies also suggest that HER2 positivity is associated with relative, although not absolute, resistance to tamoxifen therapy, and with response to anthracycline and paclitaxel therapy (Wolff et al. 2007; Pritchard et al. 2006). Therefore, women with HER2-positive tumors and positive lymph nodes are typically treated with trastuzumab and a polychemotherapy regimen containing an anthracycline and a taxane (Winer et al. 2006; NCCN 2008). In lower-risk cases (e.g., tumor <1 cm, negative

axillary nodes), however, trastuzumab therapy is not universally recommended; consideration of hormonal therapy (if tumor expresses hormone receptor) and chemotherapy is suggested in some cases (NCCN 2008).

In contrast to the large proportional and absolute benefits of tamoxifen and trastuzumab treatment seen only in women with specific tumor characteristics, the proportional benefits of adjuvant chemotherapy in disease-free and overall survival are independent and about the same for all patients, regardless of prognosis. Absolute benefits, however, vary considerably by baseline risk of recurrence (Henderson 1994), and are as high as 25% at 10 years for node-positive, ER-negative disease (Berry et al. 2006). In contrast, women at low risk with ER-positive, HER2-negative, lymph-node-negative, grade 3 tumors smaller than 2 cm in size experience only an estimated average 3% absolute risk reduction when anthracycline-based polychemotherapy is added to 5 years of tamoxifen treatment (Ravdin et al. 2001). The absolute risk reduction is even lower or negligible with non-anthracycline regimens in this population. Thus, baseline risk and likely risk reductions must be balanced against regimen toxicity by patients and physicians when deciding whether or not to prescribe adjuvant chemotherapy for node-negative disease. However, although adjuvant chemotherapy is more often prescribed for patients with a high baseline risk due to the likelihood of higher absolute benefit, patients are much more likely to accept intensive chemotherapy for even a very small chance of benefit (e.g., 1%) compared to physicians (Slevin et al. 1990).

For tamoxifen-treated, ER-positive, node-negative patients not treated with chemotherapy, the large National Surgical Adjuvant Breast and Bowel Project (NSABP) trials B-14 and B-20 demonstrated an average 10-year recurrence rate of approximately 15% (Fisher et al. 2004), suggesting that 85% of these women could be unnecessarily exposed to the toxicity of chemotherapy if it were offered to everyone. Because traditional risk evaluation is inexact, only a minority of patients with the most favorable tumor characteristics are currently excluded from recommendations to at least consider chemotherapy.

Chemotherapy Regimens for Early Breast Cancer

Regimens currently considered for adjuvant therapy of early breast cancer are CMF (cyclophosphamide, methotrexate, and 5-fluorouracil), AC (doxorubicin [an anthracycline] and cyclophosphamide) for 4 cycles, AC followed by 4 cycles of a taxane (e.g., paclitaxel or docetaxel), or TAC (docetaxel, doxorubicin, and cyclophosphamide).

CMF is an older regimen and although it improves disease-free survival in lymph node-positive women, and in lymph node-negative, ER-negative women, it appears to have no measurable benefit in lymph node-negative, ER-positive patients treated with tamoxifen (International Breast Cancer Study Group 2002).

In practice, anthracycline-based polychemotherapy regimens (i.e., containing doxorubicin or epirubicin) have largely replaced CMF in women for whom cardiotoxicity is not a concern. Several clinical trials have demonstrated that anthracycline-based regimens reduce the annual breast cancer death rate by about 38% for younger women (i.e., younger than age 50 at diagnosis) and by about 20% for those women aged 50 or older at diagnosis (EBCTCG 2005). These regimens also modestly but significantly improve outcomes (e.g., breast cancer death rate ratio 0.84, $p < 0.00001$) over those found with older regimens such as CMF. Additionally, in women with indicators of greater risk (e.g., positive lymph nodes, HER2 overexpression, negative endocrine receptors), increased dose intensity or the addition of a taxane improves outcomes (Piccart et al. 2005). The relative benefit of chemotherapy appears to be independent of tamoxifen benefit in ER-positive patients.

Adverse Effects of Chemotherapy

When the absolute benefits of chemotherapy treatment are small, the toxicities of chemotherapy may outweigh the benefits. Thus, physicians and patients need to know the frequency, duration, and severity of adverse effects when making decisions about adjuvant chemotherapy in early stage disease.

Adverse effects from chemotherapy may be short term or long term and vary according to the specific chemotherapy combination (Partridge et al. 2001). In general, regimens that contain an anthracycline are associated

with more serious grade 3–4 short-term toxicities (e.g., cardiotoxicity) than those that do not. However, the regimen of doxorubicin and cyclophosphamide is substantially shorter than other combinations, thus reducing the duration of short-term adverse effects. Women on shorter treatment regimens demonstrate a more rapid improvement in quality of life (Hurny et al. 1996).

Short-term adverse effects include nausea, vomiting, and diarrhea. In addition, fatigue is a frequently reported symptom of cancer and cancer treatment in patient surveys and has a significant impact on patients' daily lives (Portenoy et al. 1994; Vogelzang et al. 1997). The risk of thrombosis is increased during active chemotherapy treatment; the amount of increase may be as much as 5 to 11% compared to tamoxifen alone (Fisher et al. 1997; Pritchard et al. 1996). Concurrent chemohormonal therapy may be associated with a higher rate of thrombosis than chemotherapy alone (Rivkin et al. 1994). Toxicity-related deaths are rare, occurring in approximately one of every 200–500 women who are treated with adjuvant chemotherapy (Osborne and Ravdin 2000).

Additional detail on the adverse effects of chemotherapy is summarized in Appendix I.

Predicting Disease Recurrence or Chemotherapy Response

Traditional Criteria. Contemporary guidelines such as those regularly updated by the St. Gallen Consensus Panel (Goldhirsch et al. 2007), the National Comprehensive Cancer Network (NCCN; 2008), the American Society of Clinical Oncology (ASCO; Harris et al. 2007) and the Adjuvant! Online risk tool (<http://www.adjuvantonline.com/index.jsp>) estimate recurrence risk by considering criteria such as tumor size, type, grade, and histologic characteristics; hormone receptor status; and lymph node status. However, these criteria are inefficient at predicting risk of recurrence in individual patients. Reliable biomarkers for predicting risk have been lacking (Abrams 2001). Thus, to ensure that those who are likely to benefit receive chemotherapy, a large proportion of early stage breast cancer patients at low baseline risk are overtreated and exposed to unnecessary toxicity. Conversely, a small number of women who appear low risk by traditional criteria and are not recommended for chemotherapy experience disease recur-

rence and might benefit from treatment, if accurately identified.

Gene Expression Panels. Recently, several groups have identified panels of gene expression markers (“signatures”) that appear to predict the likelihood of breast cancer recurrence after surgery, radiation therapy, and hormonal therapy (for ER-positive tumors) in women with node-negative disease. If these panels are more accurate than current conventional classifiers, they could be used to aid chemotherapy decision-making, in cases for which current guidelines only advise considering chemotherapy without strong recommendations either way, without negatively affecting disease-free and overall survival outcomes.

The purpose of gene expression analysis is to evaluate which genes are actively transcribed into messenger RNA in tumor cells. Differences in the level of expression of specific gene or other nucleic acid sequences (e.g., single nucleotide polymorphisms or SNPs) can be correlated with cancer types or characteristics. Current techniques for gene expression analysis include DNA microarrays and quantitative real-time, reverse-transcriptase polymerase chain reaction (RT-PCR).

Broad-scope genomic microarrays can be used to search thousands of genetic markers for those that have strong associations with cancer types or characteristics (e.g., likelihood of recurrence or metastases) using archived tumor tissue samples from clinically representative populations with known outcomes. Another method is to select a large number of candidate genes already known to be associated with the cancer or process of interest, and using representative samples identify those with the strongest predictive value. Either method has advantages and disadvantages (see Appendix II). In both cases, the goal is to construct a smaller panel using the markers with the strongest associations with the characteristics of interest, and validate the panel in a clinical setting.

Validation of Gene Expression Panels

Validation of gene expression panels to improve risk prediction or treatment outcomes is a multistep process. Genetic test validation in general has been carefully examined in a report funded by the Centers for Disease Control and Prevention. The ACCE (analytic validity, clinical

validity, clinical utility, and ethical, legal and social implications) Model System for Collecting, Analyzing and Disseminating Information on Genetic Tests (available at <http://www.cdc.gov/genomics/activities/fbr.htm>) provides a framework that is applicable to a variety of genetic tests. The ongoing model project, Evaluation of Genomic Applications in Practice and Prevention (EGAPP), used the ACCE framework as a starting point and is establishing and evaluating a systematic, evidence-based process for assessing genetic tests and other applications of genomic technology in transition from research to practice (<http://www.egapreviews.org>).

An example of a validation sequence might be:

- Identify a starting panel of candidate genes or a DNA microarray, evaluate individual components for strength of association with outcome(s) in a clinically relevant population, and select a smaller panel of nucleic acid markers with the best results.
 - Use one set of samples (training set) to refine the panel and select clinically useful cutoff values and a second, independent set of samples (test set) to test the panel without adjusting markers or cutoff values (split-sample validation).
 - OR, use one set of samples and apply cross validation methods (e.g., leave-one-out cross validation; Simon 2006).
- Establish the specific genotyping test performance characteristics, i.e., whether the test accurately and reproducibly detects the gene markers of interest (analytic validity).
- Conduct preliminary performance study(ies) in relevant populations to evaluate test result associations with patient outcomes of interest (clinical validity); may be retrospective.
- Determine whether the use of gene expression profiling to influence management decisions regarding chemotherapy reduces adverse event rates and/or improves disease-free and overall survival (clinical utility). While epidemiologic multivariable analyses of risk ratio or hazard ratio may indicate strong statistical associations between the test result and outcomes in populations (i.e., clinical validity), the test may not adequately discriminate between individual patients who do and do not have the outcome and thus may not have clinical utility (Wald et al. 1999; Pepe et al. 2004; Ware 2006; for an example, see also Wang et al. 2006). Best

evidence for clinical utility is obtained from prospective, randomized controlled trials of standard management versus management using information from gene expression profiling. Supportive evidence of clinical utility may also be derived from retrospective analyses of archived samples. Commonly, researchers have compared receiver operating characteristic (ROC) curves, e.g., for the conventional classification method(s) with and without the addition of the gene expression panel results. However, when conventional classifiers using standard risk factors already have reasonably good discrimination, the method is relatively insensitive to the contribution of new risk markers (Pencina et al. 2008). A better method of analysis is reclassification (use the test to reclassify patients classified by conventional methods and determine whether outcomes are improved for reclassified groups). This method has the advantage of being able to consider groups that are more accurately reclassified as to outcomes with the new risk marker, as well as groups that are less accurately reclassified, as the consequences for each may differ (Pencina et al. 2008).

Regulatory Status

Of the three gene expression profiles commercially available in the U.S., only MammaPrint® has been cleared by the U.S. Food and Drug Administration (FDA; cleared February 6, 2007). All MammaPrint® tests are conducted in Agendia's CLIA-licensed central service laboratory. MammaPrint is the first cleared "in vitro diagnostic multivariate index assay" (IVDMIA). On September 7, 2006, the FDA issued new draft guidance for IVDMIA's; subsequent to extensive public commentary, a second draft guidance was issued on July 26, 2007 (<http://www.fda.gov/cdrh/oivd/guidance/1610.pdf>). In the latter document, an IVDMIA is defined as one that "1) Combines the values of multiple variables using an interpretation function to yield a single, patient-specific result (e.g., a "classification," "score," "index," etc.), that is intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment or prevention of disease, and 2) Provides a result whose derivation is non-transparent and cannot be independently derived or verified by the end user." The comment period for the second IVDMIA draft guidance has ended, but the final disposition of the guidance is unknown at the time of this writing.

Oncotype DX™ and the Breast Cancer Gene Expression Ratio are each available from only one laboratory and are not cleared by the FDA. Clinical laboratories may develop and validate tests in-house (laboratory-developed tests or LDTs; previously called “home-brew”) and market them as a laboratory service; LDTs must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Laboratories offering LDTs must be licensed by CLIA for high-complexity testing. While the FDA has technical authority to regulate LDTs, to date there has been no active oversight with the new exception of IVDMA devices.

Gene expression tests that are currently being marketed for clinical use or are being used in research protocols classify patients into disease risk or treatment response categories using algorithms that incorporate the tumor expression status of multiple genes. As such, most or all of these assays are likely to fall into the IVDMA category. Thus, it is expected that Oncotype DX™ and possibly the Breast Cancer Gene Expression Ratio would need to meet the final pre- and postmarket device requirements once the guidance document is finalized.

In general, FDA review of laboratory tests focuses largely on technical performance, assuring the reliability of test results over time. Review of clinical performance is more limited and may be based on “existing clinical data, new clinical trial data, review of information in the literature, or current clinical knowledge” (FDA Office of In Vitro Diagnostics 510(k) Workshop, April 19-20, 2005).

Methods

Search Methods

For this update, MEDLINE was searched for “Breast Neoplasms”[MeSH] AND (“Gene Expression Profiling”[MeSH] OR “Gene Expression”[MeSH]) from January 2005 through December 2007. Titles and abstracts were reviewed; full copies of potentially relevant papers published in the English language were retrieved for review. Reference lists of pertinent publications were searched for additional relevant citations. Websites of specific manufacturers were searched for publications. Keyword internet searches were used to find news articles that provided publication or meeting citations.

Study Selection

Included studies were full-length journal publications reporting on the use of gene expression panels to predict breast cancer recurrence for the purpose of identifying women with early stage breast cancer who were likely to benefit from postoperative adjuvant chemotherapy. Unpublished studies that analyzed data from published studies, for which the full presentation was available, were also accepted as evidence.

We included studies reporting evidence of statistical association between gene expression profiling results and patient outcomes (disease recurrence), which comprised the majority of the evidence. However, while associational evidence is a necessary first step and is useful for making predictions regarding populations, it is not sufficient evidence of improved risk prediction for individual patients (see Background, Validation of Gene Expression Panels). Therefore, in the absence of prospective clinical trials of clinical utility, we also searched for studies reporting either reclassification studies (individual patient risk first classified by conventional clinical criteria, then reclassified by gene expression profile result), or ROC analysis.

In the 2005 Assessment (see Medical Advisory Panel Review, following), studies that predicted response to adjuvant or neoadjuvant chemotherapy regimens were also selected and evaluated; this category of studies was omitted from this update.

Medical Advisory Panel Review

Current Assessment. This Assessment was reviewed by the Blue Cross and Blue Shield Association Medical Advisory Panel (MAP) on June 28, 2007. In order to maintain the timeliness of the scientific information in this Assessment, literature searches were performed subsequent to the Panel’s review (see “Search Methods”). If the search updates identified any additional studies that met the criteria for detailed review, the results of these studies were included in the tables and text where appropriate. There were no studies that would change the conclusions of this Assessment.

Previous Assessments. A previous Assessment on gene expression profiling for breast cancer was reviewed by the MAP on February 8, 2005. At that time, it was decided that the available evidence was insufficient to permit conclusions

regarding the use of gene expression profiling for managing breast cancer treatment. Note that the previous Assessment included a review of the 76-gene signature, which is not currently available in the U.S., and which was, therefore, omitted from the current review. The Breast Cancer Gene Expression Ratio (2-gene ratio) was not reviewed in the prior Assessment.

Formulation of the Assessment

Patient Indications

Women with lymph node-negative, hormone receptor-positive, early stage breast cancer for whom postoperative hormonal therapy is planned and additional adjuvant chemotherapy is considered. Among these patients there are two groups of greatest interest to identify: those who are defined as high risk for recurrence by conventional criteria but may actually be low risk; and those who are defined as low risk for recurrence by conventional criteria but may actually be high risk.

Technologies to Be Compared

The incremental benefit of gene expression profiling for the selection of women for postoperative adjuvant chemotherapy when added to traditional clinical and histopathologic criteria is to be determined. Several groups have published traditional risk classification criteria (see Background, Predicting Disease Recurrence or Chemotherapy Response). Treatment guidelines (NCCN 2008; Harris et al. (ASCO) 2007) currently recommend or consider adjuvant chemotherapy for most patient risk categories.

Health Outcomes

Women who are at high risk by conventional criteria but who are actually at low risk:

- **Benefits.** Use of the assay has the potential to identify more women than would be identified by traditional clinical criteria who are at low risk of distant recurrence and can safely avoid adjuvant chemotherapy and associated toxicity.
- **Harms.** If the assay incorrectly identifies some women as being at low risk who would actually benefit from chemotherapy, disease-free and overall survival may be worse for these women.

Women who are at low risk by conventional criteria but who are actually at high risk:

- **Benefits.** The assay may also identify a small subset of women who appear low risk by traditional criteria, but who are actually more likely to recur and would benefit from adjuvant chemotherapy, thereby improving their disease-free and overall survival.
- **Harms.** Assignment of high-risk status by the assay result could prompt excessively aggressive treatment choices, exposing patients to greater potential harm for uncertain benefit.

Specific Assessment Questions

1. What gene expression profiling assays are available and what is their analytic validity (technical performance)?
2. In women with early stage, primarily node-negative breast cancer, does the evidence support gene expression profiling as a clinically useful prognostic indicator for breast cancer recurrence compared to traditional clinical and histopathologic criteria, or when used in conjunction with traditional clinical and histopathologic criteria?
3. For women with low risk of breast cancer recurrence based on gene expression profiling results, who would otherwise be classified as having a higher risk based on traditional criteria, does the evidence indicate that chemotherapy can be safely avoided without negatively affecting disease-free or overall survival outcomes?
4. For women with high risk of breast cancer recurrence based on gene expression profiling results, who would otherwise be classified as having a lower risk based on traditional criteria, does the evidence indicate that chemotherapy can be recommended without negatively affecting disease-free or overall survival outcomes?

Review of Evidence

Several groups have constructed different multi-gene expression panels (“signatures”) for predicting disease recurrence (prognosis); these are now in various stages of validation testing. Only three are commercially available in the U.S.: Oncotype DX™, MammaPrint®, and the Breast Cancer Gene Expression Ratio (Table 1). Only those studies that have been published as full-text journal articles, or studies utilizing data from published studies and for which full detail on posters or presentations is available meet the evidence criteria for this Assessment.

Table 1. Commercialized Gene Expression Profiles for Predicting Breast Cancer Recurrence and Benefit from Adjuvant Chemotherapy

Panel Name (source)	Target Population	Panel Description	Specimen	Distant Recurrence Prediction	Prospective Trials in Progress
Oncotype DX™ (Genomic Health) www.genomichealth.com	Women with node-negative ER+ disease being treated with tamoxifen; 2–3% of patients had tumors larger than 4 cm	16 cancer-related gene markers and 5 reference gene markers derived from tests of 250 candidate genes. Marketed for clinical use (cost ~\$3,500); tests performed in-house.	Fixed, paraffin-embedded tumor tissue (standard surgical prep)	n=447 Training set n=668 Test set n=149 External study n=790 External study, correlation with mortality	TAILORx, partial listing of objectives: Primary: Compare the disease-free survival of women with previously resected axillary-node negative breast cancer with an Oncotype DX RS of 11–25 treated with adjuvant combination chemotherapy and hormonal therapy vs. adjuvant hormonal therapy alone. Secondary: Determine if adjuvant hormonal therapy alone is sufficient treatment (i.e., 10-year distant disease-free survival of at least 95%) for patients with an RS of ≤10.
MammaPrint® (Agendia) www.agendia.com	Women younger than age 52-61 at diagnosis with stage I or II disease. Lymph node status and treatment variable across studies.	70 gene markers selected by large, oligonucleotide microarray analysis of tumor gene expression; converted to small, custom-made microarray for commercial use; FDA approved February 6, 2007; U.S. availability pending.	Fresh tumor tissue shipped in supplied preservation fluid	n=117 Training set n=295 Test set (included 61 from training set) n=307 Test set (independent of training set) n=96 External study	MINDACT A multicenter, prospective, phase III randomized study comparing the 70-gene expression signature with a common clinical-pathological prognostic tool (Adjuvant! Online) in selecting patients for adjuvant chemotherapy in node-negative breast cancer. Patients at low risk by both MammaPrint and standard clinical-pathological criteria will not receive chemotherapy; patients at high risk by both criteria receive chemotherapy; patients with discordant criteria will be randomized to use either MammaPrint only, or standard criteria only to decide treatment.

Table 1. Commercialized Gene Expression Profiles for Predicting Breast Cancer Recurrence and Benefit from Adjuvant Chemotherapy (cont'd)

Panel Name (source)	Target Population	Panel Description	Specimen	Distant Recurrence Prediction	Prospective Trials in Progress
Breast Cancer Gene Expression Ratio	Early stage breast cancer, LN+/-, ER+, treated only with TAM	Ratio of expression of HOXB13:IL17BR genes with opposing patterns of expression, initially selected after analysis of 22,000 transcript microarray data (Ma et al. 2004)	Formalin-fixed, paraffin-embedded tumor tissue	n=60 Training set	(None found)
AviaraDx Inc. www.aviaradx.com/		Launched December 12, 2006		n=20 Test set	
Also licensed to Quest Diagnostics www.questdiagnostics.com				n=211 Validation study	
				n=58 Independent study	
				n=852 Independent study	
				n=1,252 Independent study	
				n=257 Independent study	

Abbreviations

TAILORx Trial Assigning Individualized Options for Treatment (Rx)

MINDACT Microarray for Node-Negative Disease may Avoid Chemotherapy

Other multi-gene signatures have been published and some are in various stages of development and commercialization. Appendix III, Table D summarizes panels and results for which multiple studies have been published and Appendix III, Table E lists examples of single publications. These are considered to be earlier in development than Oncotype DX™, MammaPrint®, and the Breast Cancer Gene Expression Ratio, are not commercially available in the U.S., and will not be evaluated in this Assessment.

1. What gene expression profiling assays are available and what is their analytic validity (technical performance)?

Although analytic validity will ultimately be reflected in the evidence gathered to support clinical validity and clinical utility, a separate review allows estimation of its likely contribution to any uncertainty regarding clinical validity and utility as well as information on likely routine laboratory performance. FDA-approved tests are extensively reviewed for analytic validity, but tests not subject to FDA review (see Regulatory Status) may have little available documentation of technical performance.

Oncotype DX™

Oncotype DX™ analyzes the expression of a panel of 16 marker genes and 5 control genes in formalin-fixed, paraffin-embedded tumor specimens using RT-PCR. Expression of each marker gene is normalized relative to expression of the 5 control genes, and gene results are grouped by function and/or correlated expression. The marker genes include 4 related to hormone sensitivity, 2 that measure HER2 function, 5 related to proliferative activity (e.g., Ki67), 2 related to invasion, and 3 other genes. An algorithm was developed to calculate group scores, and to calculate a final overall recurrence score (RS). Details can be found in Paik et al. (2004a).

Cronin et al. (2007) report an extensive evaluation of important components of analytic validity, with the following results:

- amplification efficiencies of 88% to 96% (for quantitative RT-PCR, ideal is 100%);
- response linearity across a wide concentration range of test RNA;
- accuracy for each gene component, reported as bias from known target values, ranging from -0.3% to 0.7%;

- precision of repeat testing for each gene component, reported as coefficients of variation, ranging from 3.2% to 5.7%;
- precision of overall RS for the same specimens including all sources of process variation was 1.5 SD, which would be a coefficient of variation of 6% at a mean RS value of 25.

In clinical practice, accuracy and reproducibility of the test alone is difficult to separate from tumor tissue variability due either to type of tissue preparation or to biologic variation within and between tumor samples. Most of the test validation has been conducted on formalin-fixed and paraffin-embedded (FFPE) samples; RNA continues to fragment into smaller pieces over time after specimens are embedded, reducing yield. Cronin et al. (2004) tested FFPE and frozen tissue prepared from the same breast tumor; tumor tissue expression profiles were similar.

Paik et al. (2004a) tested reproducibility within and between FFPE blocks. The within-block standard deviation (SD) for the RS was 0.72 RS units (95% CI: 0.55–1.0); the within-patient SD, including within-block variation, was 2.2 RS units. Habel et al. (2006) report that the RS SD between blocks from the same patient was 3.0 RS units. Among 49 patients, the concordance between results from core biopsies and resections was 86%. Paik et al. (2005) report 16 cases in which macrodissected specimens were compared to the whole section with a median difference of 3.2 RS units. Assuming a mean RS value of 25, an SD of 3 RS units corresponds to a coefficient of variation of 12%. Results for replicates run on an assay with a 12% coefficient of variation are likely to differ by 10% or more approximately 56% of the time and differ by 20% or more approximately 28% of the time (Reed et al. 2002).

Summary. Technical performance of the assay is unlikely to be a major source of variability. Tissue sampling is likely the greatest source of variability; within-patient SD is approximately 2 to 3 RS units. While tissue sampling variability may account for some of the patient variability seen in the clinical evidence summarized in the next sections, it should not add additional variability to what is already represented in the data.

MammaPrint®

van't Veer et al. (2002) developed a 70-gene expression signature from large microarray (25,000 human genes) studies of banked tumor tissue from young (i.e., younger than 55 years old), lymph-node-negative patients whose outcomes were known. The signature was chosen as the strongest set of predictors for distant metastases. The 70-gene signature (originally referred to as the “Amsterdam signature”) was later commercialized by Agendia (Amsterdam, the Netherlands) as MammaPrint® using a customized, smaller microarray that can measure expression of the 70 genes for 8 patients on a single slide (Glas et al. 2006). Agendia also established two reference samples (one high risk, one low risk) as quality controls to monitor quality and temporal stability. The test was approved by the FDA (see Background, Regulatory Status) on February 6, 2007. The intended use is to assess, in conjunction with other clinicopathologic factors, a patient's risk for distant metastasis. Patients are those who are younger than 61 years of age, have Stage I or II disease, tumor size 5 cm or smaller, and are lymph node negative. In June, Agendia received a second clearance from the FDA for its MammaPrint® sampling and room temperature shipping procedure, designed and validated to preserve RNA integrity during shipment to the Agendia laboratory.

Glas et al. (2006) reported the validation of the procedurally standardized MammaPrint® microarray test compared to the data from the original large microarray. Banked tissue samples used to develop and validate the 70-gene signature were also used in the commercial assay validation. In two sets of patients, results correlated well with the original data ($r=0.92$ and 0.88); discrepant specimens tended to be close to the classification threshold. Calculated risks of distant metastases were also similar to the original data. In a reproducibility study, 2 samples (with different prognoses) were tested repeatedly over 12 months in a single laboratory by 6 different individuals; both sample results were stable over time with coefficients of variation (CV) of 4.6% and 6.4%. Repeat analysis (40 times in 4 months) of a sample close to the classification threshold yielded a misclassification rate of 15%, similar to that expected (14%) based on the Gaussian distribution for the sample mean and the standard deviation of a quantitatively similar mean value from the reproducibility study.

Ach et al. (2007) further investigated the reproducibility of the commercialized MammaPrint® assay across three different laboratories located in the Netherlands, U.S., and France. Variability of test components (amplification/labeling, hybridization, and scanning) was separately quantified; greatest variability was seen in the amplification/labeling reaction (accounting for at most a 5% difference in the final gene expression ratio) and appeared random rather than systematic. Four tumors were repeatedly tested in all laboratories (one laboratory used different microarray and reagent manufacturing lots from the others) and compared the overall result. The percent CV of the means across laboratories for 3 samples ranged from 1.4% to 5.1%. The CV for a fourth sample was high at 34% because the sample result was much lower than the others and near 0; variation would not have resulted in different interpretations.

FDA 510(k) Summary. The FDA review (<http://www.fda.gov/cdrh/pdf7/K070675.pdf>) reported a 98.5% result accuracy and a 97.7% classification accuracy over repeated measurements. Samples near the classification threshold, comprising less than 5% of patient samples tested over 2 years, have less than 90% accuracy. The FDA also reviewed supporting clinical studies (see Review of Evidence, Key Question 2, following) and concluded that “the MammaPrint analysis is considered to be Precise, Reproducible, Sensitive, Specific, Accurate and Robust and valid for the Intended Use.”

Breast Cancer Gene Expression Ratio

The test for the Breast Cancer Gene Expression Ratio employs RT-PCR to measure the relative expression levels of HOXB13 and IL17BR genes in paraffin-embedded tumor specimens. In one report, standard curves generated on each RT-PCR plate using commercially available total human RNA control material were used to determine relative gene expression levels and to control for plate-to-plate variation (Goetz et al. 2006); in another, the results for four reference genes were used to determine relative gene expression (Ma et al. 2006).

Summary. Assay configuration and performance characteristics of the commercially available Breast Cancer Gene Expression Ratio have not been published.

2. In women with early stage, primarily node-negative breast cancer, does the evidence support gene expression profiling as a clinically useful prognostic indicator for breast cancer recurrence compared to traditional clinical and histopathologic criteria, or when used in conjunction with traditional clinical and histopathologic criteria?

Currently, risk of recurrence is estimated by evaluating several tumor characteristics, either informally or according to one of several established schemes such as the NCCN (2008) and St. Gallen (Goldhirsch 2007) risk assessment guidelines, or Adjuvant! Online (www.adjuvantonline.com). Decisions regarding chemotherapy are based on this risk assessment and patient preferences for risk versus chemotherapy toxicity. However, patients with tumors that are positive for HER2 amplification and larger than 1 cm follow a distinct treatment pathway, and are not candidates for gene expression profiling to determine treatment (NCCN 2008). Thus, gene expression profiling would have incremental predictive value if the test alone or in combination with one of the established risk assessment methods better classifies HER2-negative patients (or HER2-positive patients with tumors measuring 1 cm or smaller) according to recurrence outcome compared to current methods alone.

Oncotype DX™ (Genomic Health)

Oncotype DX™ was developed for use in women with node-negative, ER-positive breast cancer who are treated with tamoxifen (Paik et al. 2004a). Table 2 gives an overview of the published evidence on Oncotype DX, describing test development and association of gene expression results with disease recurrence.

Paik et al. (2004a) evaluated RS versus distant recurrence in a representative sample (668 of 2,617 or 25%) of patients from the tamoxifen-treated arm of National Surgical Adjuvant Breast and Bowel Project (NSABP) trial B-14 (n=2,617), for which the overall 10-year distant recurrence rate was 15%. In these patients with lymph-node-negative, ER-positive breast cancer, the categorized low-risk RS recurrence rate estimate was significantly lower than the

high-risk RS recurrence rate estimate ($p < 0.001$; Table 2). In a multivariable model, only RS and poor tumor grade were significant predictors of distant recurrence (RS hazard ratio = 2.81; 95% CI: 1.70–4.64, $p < 0.001$); neither age, tumor size, HER2 amplification, nor quantified ER protein were significant variables. Because Oncotype DX™ includes genetic evaluation of ER, HER2, and other conventional indicators of risk, it is not surprising that these indicators are not significant with RS in the model.

Only 55 of 668 patients were positive for HER2 amplification by conventional methods; HER2-positive patients had poorer distant disease-free survival than HER2-negative patients (75% vs. 86%, respectively). Fifty of 55 HER2-positive samples had high-risk RS results (0 of 55 had low-risk RS) and 28% of all high RS patients (n=179) were HER2 positive.

Habel et al. (2006) reported a case-control study derived from all node-negative breast cancer patients (age younger than 75 yrs; n=220) diagnosed from 1985–1994 at 14 Northern California Kaiser hospitals (total eligible 4,964) and not treated with adjuvant chemotherapy. HER2 status of the patients was not reported. For ER-positive, tamoxifen-treated patients, risk of death at 10 years by RS category (Table 2) was similar to results for NSABP B-14 patients (3%, 12%, and 27%, for low, intermediate, and high risk, respectively). In a multivariable analysis of ER-positive, tamoxifen-treated patients, only RS and tumor size were significant variables. The association of RS with risk of breast cancer death was stronger among tamoxifen-treated patients than among tamoxifen-untreated patients (Table 2).

Esteva et al. (2005) reported no correlation between RS and rate of distant recurrence in 149 highly selected patients with node-negative breast cancer who did not receive tamoxifen or chemotherapy. In these patients, ER, PR, and HER2 also had no prognostic value, and low nuclear grade (well-differentiated) was unexpectedly correlated with worse survival compared with higher nuclear grade tumors, suggesting that this was not a representative population.

Table 2. Summary of Published Oncotype DX™ Studies of Assay Development and Association with Disease Recurrence Outcomes

Citation	Study Description	Patient Samples				Results																				
Test Development																										
Cronin et al. 2004	Developed a high-throughput, real-time, RT-PCR method to quantify gene expression using sections of standard prep fixed, paraffin-embedded tumor tissue instead of fresh frozen tissue.	Archival paraffin-embedded breast tumor blocks and matching frozen sections from Providence St. Josephs Hospital, Burbank, CA																								
Cobleigh et al. 2005	Selected 250 “candidate genes” from the published literature, genomic databases, and experimental data for their presumed influence on breast cancer progression	Archival paraffin blocks from women with invasive breast cancer and ≥10 positive nodes but no evidence of metastatic disease from Rush Medical Center, Chicago, IL																								
Esteban et al. 2003; Cobleigh et al. 2005; Paik et al. 2003	Evaluated the relationship between candidate gene expression and breast cancer recurrence using 447 banked samples from 3 sources	NSABP B-20 ¹	n=233 LN- ER+	TAM Chemo	100% 0%																					
		Rush U Med Ctr	n=78 ≥10 LN, ER+/-	TAM Chemo	54% 80%																					
		Providence St. Josephs	n=136 LN+/- ER+/-	TAM Chemo	41% 39%																					
Paik et al. 2003; Paik et al. 2004a	Selected a panel of 16 genes strongly associated with recurrence and 5 reference genes ² ; designed an algorithm to compute a recurrence score (RS) ³ for each tumor sample	RS cutoff points based primarily on results of 234 NSABP B-20 ¹ TAM-only specimens (training set)				Final assay: the expression of each gene is measured in triplicate and normalized to the set of 5 reference genes. Calculated RS ³ results are classified as follows: – RS <18, low risk – RS 18 to <31, intermediate risk – RS ≥31, high risk																				
Risk of Distant Recurrence or Death from Breast Cancer Without Chemotherapy																										
Paik et al. 2004a	Correlated RS with distant recurrence in node-negative, TAM-treated patients from NSABP B-14 ⁴ breast cancer trial of TAM treatment Note: For all B-14 patients, average tumor size was 2.7±1.4 cm; 2% of patients had tumors >5 cm.	668 archival tumor blocks from the ER+, TAM-only arm of NSABP B-14 ⁴ (test set; B-14 trial n=2,617) Note: 675 of 754 available paraffin blocks had sufficient tumor; assay successful in 678 (99%); average tumor size in 675 patients not significantly different from 2,617 B-14 patients				<table border="1"> <thead> <tr> <th>RS Risk</th> <th>% of patients (#)</th> <th colspan="2">K-M Distant recurrence at 10 yr, % (95% CI)</th> </tr> </thead> <tbody> <tr> <td>Low (<18)</td> <td>51 (341)</td> <td>6.8</td> <td>(4.0–9.6)</td> </tr> <tr> <td>Int (18–30)</td> <td>22 (147)</td> <td>14.3</td> <td>(8.3–20.3)</td> </tr> <tr> <td>High (≥31)</td> <td>27 (180)</td> <td>30.5</td> <td>(23.6–37.4)⁵</td> </tr> <tr> <td>All</td> <td>100 (668)</td> <td>15</td> <td>(12.5 to 17.9)</td> </tr> </tbody> </table>	RS Risk	% of patients (#)	K-M Distant recurrence at 10 yr, % (95% CI)		Low (<18)	51 (341)	6.8	(4.0–9.6)	Int (18–30)	22 (147)	14.3	(8.3–20.3)	High (≥31)	27 (180)	30.5	(23.6–37.4) ⁵	All	100 (668)	15	(12.5 to 17.9)
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Table 2. Summary of Published Oncotype DX™ Studies of Assay Development and Association with Disease Recurrence Outcomes. (cont'd)

Citation	Study Description	Patient Samples	Results															
Risk of Distant Recurrence or Death from Breast Cancer Without Chemotherapy (cont'd)																		
Esteva et al. 2005	Correlated RS with distant recurrence using archival tumor tissue from patients with LN-, invasive breast cancer	149 evaluable of 220 archival specimens meeting eligibility criteria: no TAM or chemo; LN-; ER+/-; median follow-up 18 years	No significant correlation between age, tumor size, or RS and distant recurrence-free survival															
Habel et al. 2006	Determined the degree to which RS predicts the risk of breast cancer-specific mortality in a nested case-control study; cohort was all Northern California Kaiser Permanente tumor registry members with LN-invasive breast cancer from 1985 to 1994	Cases (n=220 evaluable) were patients whose first event was death from breast cancer; up to 3 matched (age, race, year of diagnosis, TAM treatment) living controls (n=570 evaluable) randomly selected for each case; all LN-, diagnosed at age <75 yr, no chemo; 33% of cases and 37% of controls TAM-treated	<table border="1"> <thead> <tr> <th>RS Risk</th> <th colspan="2">10-yr Absolute Risk of Death, % (95% CI)</th> </tr> <tr> <td></td> <th>ER+, TAM-treated</th> <th>ER+, No TAM</th> </tr> </thead> <tbody> <tr> <td>Low (<1-8)</td> <td>2.8 (1.7-3.9)</td> <td>6.2 (4.5-7.9)</td> </tr> <tr> <td>Int (18-30)</td> <td>10.7 (6.3-14.9)</td> <td>17.8 (11.8-23.3)</td> </tr> <tr> <td>High (≥31)</td> <td>15.5 (7.6-22.8)</td> <td>19.9 (14.2-25.2)</td> </tr> </tbody> </table> <p>ER+ patients, TAM-treated (n=255): multivariable analysis (tumor size, grade, RS) showed that RS (continuous variable, RR 5.3, 95% CI: 1.6-17.2) was a significant predictor of breast cancer death</p> <p>ER+ patients, no TAM (n=361): similarly, RS was a significant predictor of breast cancer death (RR 2.4, 95% CI: 1.1-5.2)</p> <p>ER- patients (n=108): similarly, RS significantly predicted breast cancer death (RR 6.2, 95% CI: 1.2-31.8) but results based on small numbers</p>	RS Risk	10-yr Absolute Risk of Death, % (95% CI)			ER+, TAM-treated	ER+, No TAM	Low (<1-8)	2.8 (1.7-3.9)	6.2 (4.5-7.9)	Int (18-30)	10.7 (6.3-14.9)	17.8 (11.8-23.3)	High (≥31)	15.5 (7.6-22.8)	19.9 (14.2-25.2)
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Abbreviations

RT-PCR: reverse transcriptase polymerase chain reaction; NSABP: National Surgical Adjuvant Breast and Bowel Project; LN: lymph node; TAM: tamoxifen; chemo: chemotherapy; RS: recurrence score; K-M: Kaplan Meier; ER: estrogen receptor; Int: intermediate; RR: relative risk; n: number; -: negative; +: positive.

¹ NSABP B-20, a multicenter study that involved more than 2,300 women with node-negative, ER+ disease, found that that adding either methotrexate and 5-fluorouracil (MF) or cyclophosphamide and MF (CMF) to TAM resulted in a greater benefit than TAM alone. After 5 and 8 years of follow-up, disease-free and overall survival was significantly greater with TAM plus chemotherapy (84% vs. 77%, $P < 0.001$, for disease-free survival; 92% vs. 88%, $P = 0.018$, for overall survival). (Fisher et al. 2001)

² Genes associated with proliferation: Ki-67, STK15, Survivin, Cyclin B1, MYBL2. Genes associated with the estrogen receptor: ER, PGR, Bcl2, SCUBE2. Genes associated with cancer invasion: Stromolysin 3, Cathepsin L2. Genes associated with the HER2 amplicon: GRB7, HER2. Other genes: GSTM1, CD68, BAG1. Reference genes: beta-actin, GAPDH, RPLPO, GUS, TFRC.

³ RS calculated as follows: Unscaled RSU = $+0.47 \times \text{GRB7 group score} - 0.34 \times \text{ER group score} + 1.04 \times \text{proliferation group score} + 0.10 \times \text{invasion group score} + 0.05 \times \text{CD68} - 0.08 \times \text{GSTM1} - 0.07 \times \text{BAG1}$. A plus sign indicates that increased expression is associated with an increased risk of recurrence; minus sign indicates association with a decreased risk of recurrence.

RS is rescaled from the unscaled RS, as follows: $\text{RS} = 0$ if $\text{RS}_U < 0$; $\text{RS} = 20 * (\text{RS}_U - 6.7)$ if $0 \leq \text{RS}_U \leq 100$; and $\text{RS} = 100$ if $\text{RS}_U > 100$.

⁴ NSABP B-14 randomized 2892 women with node-negative, ER+ disease to either placebo or tamoxifen. Enrolled patients were approximately 88% white, <70 years old, and had a life expectancy of at least 10 years. Throughout 15 years of follow-up, women on average had significantly better outcomes after tamoxifen treatment than after placebo. Recurrence-free survival was 78% vs. 65%, $p < 0.0001$ and overall survival was 71% vs. 65%, $p = 0.0008$. (Fisher et al. 2001)

⁵ $p < 0.001$ compared to the low-risk category.

In a 2004 San Antonio Breast Cancer Symposium poster presentation, Paik et al. (2004b¹) classified the 668 NSABP B-14 patient samples evaluated by Paik et al. (2004a) in the validation study using the NCCN 2004 risk assessment (which included HER2 as a risk stratification criterion), then reclassified by Oncotype DX™ results. (Table 3). Similarly, Bryant (2005) initially classified the same patients using Adjuvant! Online, then reclassified by Oncotype DX™ results. The 10-year outcomes for each reclassified group compared to outcomes for the initial classification indicate that Oncotype DX™ adds additional risk information to the conventional clinical classification of individual high-risk patients. A subset of patients who would otherwise be recommended for chemotherapy, are actually at lower risk of recurrence (average 7–9% risk at 10 years; upper 95% confidence interval [CI]

limits, 11–14%). Oncotype DX™ testing also identifies a subset of conventionally classified low-risk patients who are reclassified at higher risk of recurrence. However, due to wide confidence intervals, it is not clear that all reclassified higher-risk individuals would realize a net benefit from chemotherapy.

In the study by Habel et al. (2006), cross-classification of tamoxifen-treated patients by RS and tumor size/grade suggested that tumor size and grade provide additional risk information after RS low-risk classification. However, there is no information on outcomes after cross-classification.

Summary. Retrospective epidemiologic analyses indicate strong, independent associations between Oncotype DX™ RS result and distant disease recurrence or death from breast cancer

Table 3. Distant Recurrence Outcomes After Classification by Conventional Risk Criteria and Reclassification Using Oncotype DX Recurrence Score (RS) Risk

Conventional Classification	n	Survival or Recurrence Rates	95% CI ¹
% Distant Recurrence-Free at 10 Years¹			
Paik et al. 2004b			
NCCN low risk	53	93	86–100
RS low risk	38	100	Not reported ²
RS intermediate risk	12	80	59–100
RS high risk	3	56	13–100
NCCN high risk	615	85	Not reported ³
RS low risk	300	93	89–96
RS intermediate risk	137	86	80–92
RS high risk	178	70	62–77
% Distant Recurrence at 10 Years¹			
Bryant 2005			
Adjuvant! Online low risk	354	8.4	Not reported
RS low risk	216	5.6	(2.5–9)
RS intermediate-high risk	138	12.9	(7–19)
Adjuvant! Online intermediate-high risk	314	22.2	Not reported
RS low risk	122	8.9	(4–14)
RS intermediate-high risk	192	30.7	(24–38)

¹ Estimated from poster or presentation graphs

² ≥ 99 to 100; actual limits obscured on graph

³ ≥ 81 to ≤ 88 ; actual limits obscured on graph

* Full poster presentation obtained by personal communication from Genomic Health, December 18, 2004. The results of this study are also partially published in Hornberger et al. (2005). In this economic analysis, reclassification data were used to model the likely change in NCCN risk classification after Oncotype DX™ testing, and the likelihood of subsequent changes in management decisions. Reclassification analysis was also done using the 2005 St. Gallen risk assessment criteria (Goldhirsch et al. 2005); however, these criteria did not include HER2 as a risk stratification criterion, so this part of the study is not discussed.

in one study of samples from a well-controlled trial and another in a community-based population. Reclassification analyses conducted on the data from the first of these two studies suggest that the test provides risk information in addition to conventional criteria primarily for conventionally classified high-risk women who are reclassified by RS to low risk.

MammaPrint®

van de Vijver et al. (2002) studied the association of disease recurrence with the 70-gene signature results on banked tumor specimens from a consecutive series of 295 breast cancer patients. (Table 4). On average, these women were at higher risk for recurrence than those enrolled in the Oncotype DX™ studies, as women with positive lymph nodes, with hormone-receptor-negative tumors, and who did not receive tamoxifen for hormone-receptor-positive tumors were included. Patients with good prognosis gene expression signatures had significantly better 5- and 10-year distant disease-free survival than did patients with poor prognosis gene expression signatures (Table 4). In a multivariable analysis, poor gene expression signature, large tumor size, and no adjuvant chemotherapy were the strongest predictors of distant metastases (Table 4). No ROC or reclassification analysis was reported on these data. ROC analysis performed on the data used to develop the assay indicated that area under the curve (AUC) calculations for the gene signature were no better than those for 2 of 3 conventional classifiers (Eden et al. 2004).

A potential limitation of the van de Vijver et al. (2002) study was the inclusion of 61 samples that were also used to develop the assay. Therefore, an independent, multicenter validation study was conducted by the translational research network of the Breast International Group (TRANS-BIG; Buyse et al. 2006) in patients with node-negative disease. The 70-gene signature was determined for frozen archival tumor specimens from node-negative patients who had received no systemic adjuvant therapy. In 302 evaluable patients with a median follow-up of 13.6 years, the overall rate of distant metastasis was 25%. The 70-gene signature hazard ratios for time to distant metastases and overall survival, adjusted in individual analyses for commonly used clinical prediction algorithms, were statistically significant (Table 4). In the reverse analysis, when the Adjuvant! Online hazard ratios were adjusted for the gene signature, neither

disease-free nor overall survival hazard ratios were significant (data not shown). However, ROC analysis resulted in only a slightly higher area under the curve for the gene signature than for the Adjuvant! Online risk assessment (Table 4). When patients were first classified by the Adjuvant! Online risk assessment tool, then by the 70-gene signature, low-risk patients by the 70-gene signature in either Adjuvant! Online risk group had 10-year disease-free survival rates of 88–89%, with lower confidence limits of 74–77%.

The adjusted gene signature hazard ratios reported by Buyse et al. (2006) are statistically significant, but lower than those reported by van de Vijver et al. (2002). However, median follow-up time in the earlier study was only 6.7 years. Buyse et al. (2006) calculated gene signature hazard ratios at yearly intervals, and found higher hazard ratios in the first 5 years of follow-up.

None of the ER-positive patients reported by Buyse et al. (2006) received hormonal therapy. Espinosa et al. (2005) used a different technology to measure the 70-gene signature in 96 patients with early stage disease, the majority of whom received chemotherapy; most ER-positive patients received tamoxifen (Table 4). The adjusted gene signature hazard ratio was highly significant at 6.3 (95% CI: 1.28–31.1). However, in women older than 52 years, overall survival was not different between good and poor prognosis groups.

Summary. MammaPrint® gene signature hazard ratios for distant metastases suggest that the test provides information in addition to conventional classification criteria; the strongest associations appear in the first 5 years of follow-up. However, ROC analysis in an independent multicenter validation study suggests only slightly improved predictive accuracy for time to distant metastases with MammaPrint® compared to other conventional criteria. In one study, after Adjuvant! Online risk classification, patients reclassified as low risk by the 70-gene signature in either Adjuvant! Online risk group had 10-year disease-free survival rates of 88–89%, with lower confidence limits of 74–77%.

Breast Cancer Gene Expression Ratio

Ma et al. (2004) identified a composite predictor of 2 genes with opposing patterns of expression: the ratio of HOXB13 to IL17BR gene expression levels (Table 5). The test was

Table 4. Summary of Published MammaPrint® Studies

Citation	Study Description	Patient Samples	Results														
Test Development																	
van't Veer et al. 2002	Developed a 70-gene prognostic signature for distant metastases using 25,000-gene microarrays and supervised classification analysis	98 lymph-node-negative breast cancers from patients younger than 55 years of age at diagnosis, 34 from patients who developed distant metastases within 5 years, 44 from patients who were disease free for at least 5 years, and 20 from BRCA mutation carriers. (Training set)	<p>In unsupervised clustering analysis, expression of approximately 5,000 genes differed at least 2-fold across samples. Supervised classification of these 5,000 genes resulted in 231 genes significantly associated with disease outcome; cross-validation was used to narrow the gene number to the final 70. "Good" prognosis patients had 90% probability of recurrence-free survival.</p> <p>In a separate analysis of 19 tumor samples from young, node-negative women, the 70-gene predictor was strongly associated with distant metastasis ($p=0.0018$).</p> <p>Eden et al. (2004) performed ROC analysis on this data set: AUCs for the gene signature were no better than those for 2 of 3 conventional classifiers.</p>														
Risk of Distant Recurrence or Death from Breast Cancer Without Chemotherapy																	
van de Vijver et al. 2002	Evaluated 70-gene signature for predicting distant metastases	Banked tumor specimens from a consecutive series of 295 women with breast cancer; 49% were LN+; 77% ER+ but only 13% treated with TAM; 63% received adjuvant chemotherapy; all patients were younger than 53 years at diagnosis. (Test set. Note: included 61 of the 78 training set patients.)	<table border="1"> <thead> <tr> <th rowspan="2">Prognosis signature</th> <th rowspan="2">n</th> <th colspan="2">% Distant disease-free survival (95% CI)</th> </tr> <tr> <th>at 5 yr</th> <th>at 10 yr</th> </tr> </thead> <tbody> <tr> <td>Good</td> <td>115</td> <td>94.7 (90.6–98.8)</td> <td>85.2 (78.7–91.7)</td> </tr> <tr> <td>Poor</td> <td>180</td> <td>60.5¹ (53.4–67.6)</td> <td>50.6¹ (43.3–57.9)</td> </tr> </tbody> </table> <p>HR for distant metastases: 4.6; 95% CI: 2.3–9.2; $p<0.001$ Adjusted for age, LN ($p=0.01$) and ER status, tumor size ($p<0.001$), tumor grade, vascular invasion, mastectomy, chemotherapy ($p<0.001$), hormonal therapy; p-values not shown are not significant</p>	Prognosis signature	n	% Distant disease-free survival (95% CI)		at 5 yr	at 10 yr	Good	115	94.7 (90.6–98.8)	85.2 (78.7–91.7)	Poor	180	60.5 ¹ (53.4–67.6)	50.6 ¹ (43.3–57.9)
Prognosis signature	n	% Distant disease-free survival (95% CI)															
		at 5 yr	at 10 yr														
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Poor	180	60.5 ¹ (53.4–67.6)	50.6 ¹ (43.3–57.9)														

Table 4. Summary of Published MammaPrint® Studies (cont'd)

Citation	Study Description	Patient Samples	Results		
Risk of Distant Recurrence or Death from Breast Cancer Without Chemotherapy (cont'd)					
Buyse et al. 2006	Multicenter, multinational collaborative evaluation of the prognostic value of the 70-gene signature using the MammaPrint® custom array. Conducted under the aegis of the TRANSBIG consortium.	Tumor samples and clinical data were gathered from 5 centers; eligible patients were younger than 61 yrs at diagnosis, diagnosed before 1999 with LN-, ≤5 cm breast cancer and did not receive TAM or chemotherapy. Of 326 patients initially enrolled, 302 were evaluable. (Test set)	Adjusted for:		
				Adjusted gene expression HR (95% CI)	
				Time to distant metastases	Overall survival
			St. Gallen	2.15 (1.25–3.71)	2.69 (1.53–4.73)
			NPI	2.15 (1.19–3.92)	2.89 (1.58–5.29)
			Adjuvant!	2.13 (1.19–3.82)	2.63 (1.45–4.79)
		ROC Area under the curve for:			
		Time to distant metastases	Overall survival		
	70-gene signature	0.681	0.648		
	Adjuvant!	0.659	0.576		
Espinosa et al. 2005	Used quantitative RT-PCR (in place of microarrays) to measure 70-gene expression signature	Fresh frozen archived specimens from 96 patients (median age 57 years) with Stage I-II disease, surgery between 1991-7. 50% were LN-, 75% were hormone receptor+, 63% had tumors >2 cm. 74% received chemotherapy, 80% of hormone receptor+ received TAM.	Multivariable analysis for overall survival, adjusted for age, hormone receptor and LN status, and gene signature: LN status: HR=1.2, 95% CI: 1.09–1.36 Gene signature: HR=6.3, 95% CI: 1.28–31.07 (no other significant variables)		

Abbreviations

ER: estrogen receptor; HR: hazard ratio; LN: lymph node; NPI: Nottingham Prognostic Index; RT-PCR: real-time polymerase chain reaction; TAM: tamoxifen; TRANSBIG: Translating molecular knowledge into early breast cancer management; Breast International Group

¹ p<0.001 compared to good prognosis group

Table 5. Summary of Published Breast Cancer Gene Expression Ratio Studies

Citation	Study Description	Patient Samples	Results
Test Development			
Ma et al. 2004	Gene expression profiling of early stage breast cancer using large microarray; select and test composite gene signature associated with disease recurrence	<p>Training set: 60 women with ER+ early stage breast cancer treated only with TAM</p> <p>Test set: 20 additional women with ER+ early stage breast cancer treated with TAM</p>	<p>Training set: Gene expression profiling using 22,000-gene microarray; identified composite predictor of 2 genes with opposing patterns of expression; ratio of HOXB13:IL17BR had greatest AUC in ROC analysis.</p> <p>Training set: 2-gene expression ratio independently predicted disease-free survival in multivariate analysis (OR=7.3; 95% CI: 2.1–26.3, p=0.0022).</p> <p>Test set: Higher HOXB13 expression correlated with recurrence outcome (p=0.024); recurrence was correctly predicted in 16 out of 20 patients.</p>
Risk of Distant Recurrence or Death from Breast Cancer Without Chemotherapy			
Goetz et al. 2006	Tested 2-gene expression ratio on samples from North Central Cancer Treatment Group trial 89-30-52	Archived tumor blocks from 211 women with ER+ breast cancer enrolled in the TAM-only treatment arm	<p>In the LN-negative cohort (n=130), patients with 2-gene expression ratio greater than the identified cutoff had worse disease-free survival in multivariable analysis: HR 2.0, 95% CI: 1.2–3.6). Patients with 2-gene expression ratio less than the cutoff (lower risk) had a 10-year recurrence rate of about 25%.</p> <p>2-gene ratio not associated with survival in LN-positive cohort.</p>
Reid et al. 2005	Tested 2-gene expression ratio on banked samples from the Istituto Nazionale Tumori	58 patients with ER+, mostly LN+ breast cancer, treated with TAM	No association between 2-gene expression ratio and distant recurrence.
Ma et al. 2006	Tested 2-gene expression ratio on samples from Baylor College Breast Center Tumor Bank	Archived tumor blocks from 286 TAM-treated and 566 TAM-untreated women with breast cancer and no distant metastases	<p>Randomly chosen training set (n=205) of untreated ER+, LN- samples used to determine cutoff for low vs. high risk of recurrence.</p> <p>In the test set (rest of untreated plus treated ER+, LN- patients, n=225), the 2-gene ratio predicted recurrence in multivariate analysis (HR 3.9; 95% CI: 1.5–10.3); ratio predicts outcome on a continuous scale.</p> <p>2-gene ratio predicted recurrence in ER+, LN-, TAM-treated subset (p=0.026 by Kaplan-Meier analysis). Low-risk patients had a 10-year recurrence rate of about 17%. No information on untreated subset.</p> <p>2-gene ratio did not predict recurrence in LN+ subset.</p>

Table 5. Summary of Published Breast Cancer Gene Expression Ratio Studies (cont'd)

Citation	Study Description	Patient Samples	Results
Risk of Distant Recurrence or Death from Breast Cancer Without Chemotherapy (cont'd)			
Jansen et al. 2007	Retrospective evaluation of 2-gene expression ratio on samples from Erasmus Medical Clinic, Netherlands	1,252 frozen tumor samples from patients with primary operable breast cancer who entered the clinic between 1979 and 1996	<p>2-gene ratio predicted recurrence-free survival in 468 ER+, LN-, (no TAM) patients: multivariable HR=1.74 (95% CI: 1.17-2.59; p=0.006) controlled for age, menopausal status, tumor size, lymph node status, grade, ER, and PR levels</p> <p>2-gene ratio predicted progression free survival in 193 ER+, LN+/- patients treated with TAM for recurrence: multivariate HR=1.95 (95% CI: 1.39-2.73; p<0.001) controlled for age, menopausal status, disease-free survival, site of relapse, ER and PR levels</p>
Jerevall et al. 2008	Retrospective evaluation of 2-gene expression ratio on samples from randomized controlled trial conducted at 2 medical centers in Sweden	257 postmenopausal patients from RCT comparing 2 vs. 5 years of tamoxifen; tumors ER+/-, LN+/-	<p>2-gene ratio predicted improved recurrence-free survival after prolonged hormonal therapy, recurrence RR=0.39, p=0.030 in patients with low ratios.</p> <p>No difference in recurrence-free survival between 2 vs. 5 yrs TAM for patients with high ratios.</p> <p>Results were statistically significant in multivariate analysis, after adjusting for tumor size, nodal status, and PR.</p>

commercialized by AviaraDx Inc., licensed to Quest Diagnostics, and launched in December, 2006. In October 2007, AviaraDx's in-house laboratory was accredited by the College of American Pathologists (which has deemed authority under CLIA for laboratory inspections) and there the test is offered under the name "H/I." According to test information from Quest Diagnostics the Breast Cancer Gene Expression Ratio is indicated for "treatment-naïve individuals with ER-positive/lymph node-negative breast cancer" (http://www.questdiagnostics.com/hcp/intguide/jsp/showintguidepage.jsp?fn=HematOnc/Breast/BC_GeneExpression/TS_BC_Gene_Expression.htm). However, most studies, including the training set, focus on patients who received tamoxifen (Table 5). Therefore, this analysis assumes that the indicated population has received tamoxifen.

Goetz et al. (2006) tested the 2-gene expression ratio on 211 tumor blocks from the tamoxifen-only treatment arm of the North Central Cancer Treatment Group trial 89-30-52 (Table 5). In the lymph-node-negative cohort, patients with a low risk 2-gene expression ratio had a 10-year recurrence rate of about 25%. High-risk results were significantly associated with poorer disease-free survival (HR 2.0; 95% CI: 1.2–3.6). Ma et al. (2006) studied archived tumor blocks from 286 tamoxifen-treated and 566 tamoxifen-untreated women with breast cancer and no distant metastases. In lymph-node-negative, ER-positive patients, the 2-gene expression ratio significantly and independently predicted recurrence (adjusted HR 3.9; 95% CI: 1.5–10.3). The association was also significant for the tamoxifen-treated subset ($p=0.026$ by Kaplan-Meier analysis); in this subset low-risk patients had a 10-year recurrence rate of about 17%.

Ma et al. (2006) reported that the 2-gene ratio did not predict recurrence in the lymph-node-positive subset of patients. Similarly, Reid et al. (2005) found no association between the 2-gene expression ratio and distant recurrence in samples from 58 patients with mostly lymph-node-positive disease.

Jansen et al. (2007) measured HOXB13 and IL17BR expression levels using a different assay, but in concordance with Ma et al. (2006) transformed results and generated a composite index for comparison. Samples were archived from patients with primary operable breast cancer and for whom follow-up data were

available. The 2-gene ratio was significantly associated with recurrence-free survival in patients treated with tamoxifen (adjusted HR=1.95; 95% CI: 1.39–2.73; $p<0.001$) and those not treated with tamoxifen (adjusted HR=1.74; 95% CI: 1.17–2.59; $p=0.006$). The recurrence-free survival rate of low-risk patients was not reported.

Finally, Jerevall et al. (2008) reported that the 2-gene ratio predicted benefit of prolonged endocrine therapy (5 years vs. 2 years) for low-risk but not for high-risk patients.

None of these studies reported ROC or reclassification analyses.

Summary. The Breast Cancer Gene Expression ratio is significantly and independently associated with disease-free survival particularly in lymph-node-negative, ER-positive, tamoxifen-treated patients with breast cancer. Where reported, however, the recurrence rate of low-risk patients was 17–25%. No ROC analyses or reclassification studies compared risk classification by conventional methods to that by Breast Cancer Gene Expression Ratio.

5. For women with low risk of breast cancer recurrence based on gene expression profiling results, who would otherwise be classified as having a higher risk based on traditional criteria, does the evidence indicate that chemotherapy can be safely avoided without negatively affecting disease-free or overall survival outcomes?

No prospectively gathered evidence on the outcomes of using gene expression testing to guide chemotherapy decisions exists. Prospective clinical trials are currently underway for Oncotype DX™ and MammaPrint® (Table 1 and Discussion).

Oncotype DX™

For RS low-risk NSABP B-14 patients with lymph-node-negative, ER-positive, tamoxifen-treated but chemotherapy-naïve breast cancer, Paik et al. (2004a) estimated the 10-year risk of distant recurrence to be about 7%, with an upper confidence limit of nearly 10%. Habel et al. (2006) estimated the 10-year risk of death from breast cancer to be about 3% (95% CI: 1.7–3.9) in a similar but community-based population. For women at this baseline risk, the average absolute risk reduction obtained from adding anthracycline-based chemotherapy to

tamoxifen is no more than 1–2% (Ravdin et al. 2001). Thus, if the RS risk predictor is accurate and adds significant information to conventional risk assessment, then it is likely that women who are low risk by RS, even if high risk by conventional criteria, would benefit little from chemotherapy and toxicity may be the more important concern. This hypothesis is supported by Paik et al. (2004b), and Bryant (2005) who showed in the Paik et al. (2004a) population that women at high or intermediate risk by conventional criteria (and who might therefore be recommended for chemotherapy) but low risk by RS have a risk of distant recurrence of 7–9% with upper confidence limits of 11–14% (Table 3).

Paik et al. (2006) evaluated RS as a predictor of responsiveness to chemotherapy using banked specimens from the already completed NSABP B-20 trial (Table 6). Available samples from the randomized tamoxifen plus chemotherapy-treated arms were compared to those from the tamoxifen-only arm and gene expression signatures were correlated to chemotherapy benefit with respect to 10-year distant recurrence-free survival. No information on HER2 status was provided. Patients in the low-risk RS category derived no statistically significant benefit from chemotherapy (RR for distant recurrence at 10 years=1.31; 95% CI: 0.46–3.78). However, the confidence interval is wide, and includes the possibility of a result favoring chemotherapy. Furthermore, there were no similar analyses done using patients risk-stratified by conventional criteria for comparison.

Paik et al. (2006) also evaluated RS as a continuous variable for interaction with chemotherapy. After adjusting for age, tumor size, tumor grade, and site, the result bordered on significance at $p=0.035$ to 0.068 . Likelihood ratio tests for the interaction between each variable and chemotherapy (separate tests; unadjusted) suggested that the RS and chemotherapy interaction was the only significant one ($p=0.038$) but there were insufficient data to place all variables and interaction terms in

the same model to confirm.* In addition, this study used samples from the tamoxifen-only arm of the NSABP B-20 trial that were also used to select the genes used in the Oncotype DX™ assay, and to design the algorithm for the RS. It is strongly recommended that validation studies not use specimens that were used to design a test to avoid overfitting the data (Simon 2006). However, as Paik et al. (2006) note, B-20 tamoxifen-only recurrence rates by RS category were similar to those from NSABP B-14 tamoxifen-only arm (not used in assay design).

Summary. Reclassification data from two studies suggest that Oncotype DX™ adds additional risk information to the conventional high-risk category, identifying a subset of patients who are at lower risk of recurrence with at most (i.e., upper confidence limits) a 10–14% risk of distant recurrence at 10 years. Another study reported that patients in the Oncotype DX™ low-risk category derived no apparent benefit from chemotherapy, although the confidence interval was wide and included the possibility of a result favoring chemotherapy. This latter study did not include similar analyses of patients risk-stratified by conventional criteria for comparison.

MammaPrint®

Summary. Study populations used to develop and validate MammaPrint® were highly heterogeneous with respect to baseline risk; average 10-year disease-free recurrence in low-risk patients by MammaPrint® was approximately 85–88% in two studies with a lower confidence limit of 74–79%. At this baseline risk, average absolute risk reduction from chemotherapy is about 3–4%. AUC analysis does not suggest that MammaPrint® contributes significant risk information in addition to conventional classification criteria. In one study, after Adjuvant! Online risk classification, patients reclassified as low risk by the 70-gene signature in either Adjuvant! Online risk group had 10-year disease-free survival rates of 88–89%, with lower confidence limits of 74–77%.

*Additional concerns with the multivariate analysis. Continuous clinical variables (e.g., age, quantitative ER) were dichotomized in the model, but RS was continuous. This maximizes the information contained in RS, but likely minimizes the information contained in the clinical variables and thus the analysis may favor RS. Suggestions for improving the modeling include:

- Exploratory models that each include RS, RS interaction with chemotherapy, and one of the clinical variables (continuous, if applicable) plus the clinical variable-chemotherapy interaction term.
- Construction of a composite clinical variable, derived from all clinical variables (input data being continuous, where applicable); explore the interaction of this composite variable with chemotherapy alone and with RS and RS-chemotherapy interaction.

Table 6. Summary of Oncotype DX™ Study of RS Risk and Chemotherapy Outcome

Citation	Study Description	Patient Samples	Results		
Paik et al. 2006	Determined whether the RS predicts the magnitude of chemotherapy benefit using NSABP B-20 ¹ archival samples from both chemo- and chemo+ (MF or CMF) study arms. Note: for all B-20 patients, 20% had tumors ≤1 cm, 4% had tumors >4 cm.	Archival paraffin blocks with sufficient cancer tissue available for 670 patients, 227 of 770 TAM-only and 424 of 1,529 TAM+chemo; 651 evaluable by Oncotype DX™; average tumor size similar for evaluable vs. all clinically eligible patients	RS Risk Group	% of patients	RR of distant recurrence at 10 yr, chemo vs. none
			Low (<18)	54	1.31 (95% CI: 0.46–3.78)
			Int (18–30)	21	0.61 (95% CI: 0.24–1.59)
			High (≥31)	25	0.26 (95% CI: 0.13–0.53)
			Likelihood of distant recurrence fit as a linear function of RS: RS is a continuous function in both TAM and TAM+chemo arms; there is no clear RS cutoff below which there is no demonstrable benefit from chemotherapy.		

Abbreviations

NSABP: National Surgical Adjuvant Breast and Bowel Project; TAM: tamoxifen; chemo: chemotherapy; RS: recurrence score; ER: estrogen receptor; Int: intermediate; RR: relative risk; -: negative; +: positive

¹ NSABP B-20, a multicenter study that involved more than 2,300 women with node-negative, ER+ disease, found that that adding either methotrexate and 5-fluorouracil (MF) or cyclophosphamide and MF (CMF) to TAM resulted in a greater benefit than TAM alone. After 5 and 8 years of follow-up, disease-free and overall survival was significantly greater with TAM plus chemotherapy (84% vs. 77%, $P<0.001$, for disease-free survival; 92% vs. 88%, $P=0.018$, for overall survival). (Fisher et al. 2001)

Breast Cancer Gene Expression Ratio

Summary. Among lymph-node-negative, ER-positive, tamoxifen-treated patients in 2 studies, patients who were low risk by the 2-gene expression ratio had average 10-year recurrence rates of about 17–25%. At this baseline risk, average absolute risk reduction from chemotherapy is about 3–4%. No ROC or reclassification analyses show whether the Breast Cancer Gene Expression Ratio better classifies conventionally classified high-risk patients according to recurrence outcomes. No published studies retrospectively evaluated the ability of the Breast Cancer Gene Expression Ratio to predict chemotherapy benefit in comparison to conventional criteria.

4. For women with high risk of breast cancer recurrence based on gene expression profiling results, who would otherwise be classified as having a lower risk based on traditional criteria, does the evidence indicate that chemotherapy can be recommended without negatively affecting disease-free or overall survival outcomes?

No prospectively gathered evidence on the outcomes of using gene expression testing to guide chemotherapy decisions exists. Prospective clinical trials are currently underway for Oncotype DX™ and MammaPrint® (Table 1 and Discussion).

Oncotype DX™

See Oncotype DX™ text for Assessment question 3 for additional study description.

For RS high risk NSABP B-14 patients with lymph node-negative, ER-positive, tamoxifen-treated but chemotherapy-naïve breast cancer, Paik et al. (2004a) estimated the 10-year risk of distant recurrence to be about 30%, with a 95% confidence interval of 24–37%. Habel et al. (2006) estimated the 10-year risk of death from breast cancer to be about 16% (95% CI: 8–23) in a similar but community-based population. Paik et al. (2004b) performed a reclassification analysis on the Paik et al. (2004a) data (n=668) and found 3 women at low risk by conventional criteria who were high risk by RS; however the 95% confidence interval for 10-year distant disease-free recurrence was 13–100% (Table 3). In this same analysis, 12 women who were low risk by conventional criteria but intermediate risk by RS had an estimated 80% distant

recurrence-free survival (95% confidence interval, 59–100%). This analysis suggests that Oncotype DX™ testing identifies a small number of women at high risk of recurrence who would not otherwise be identified, and who are more likely to benefit from chemotherapy. However, small numbers make this conclusion uncertain. Bryant (2005) reported low-risk patients by Adjuvant! Online reclassified as intermediate to high risk by RS had a 10-year recurrence rate of about 13% (95% CI: 7–19), but because the intermediate- and high-risk RS groups were combined, these data are less helpful.

Paik et al. (2006) reported that patients in the high-risk RS category derived a statistically significant benefit from chemotherapy (RR for distant recurrence at 10 years=0.26, 95% CI: 0.13–0.53). Results suggested a chemotherapy benefit for patients in the intermediate RS category, but the confidence interval included the possibility of no benefit. Results were similar for any (locoregional and/or distant) recurrence-free and overall survival. However, there were no similar analyses done using patients risk-stratified by conventional criteria for comparison.

Summary. Reclassification data from one study suggest that Oncotype DX™ adds additional risk information to the conventionally classified low-risk category, identifying a very small subset of patients who are at higher risk of recurrence. However, due to the small numbers the confidence interval for 10-year distant disease-free recurrence for this patient subset is wide and includes 100%. A second study showed that patients in the Oncotype DX™ high-risk category derived significant benefit from chemotherapy, with a confidence interval of 0.13–0.53 for the relative risk of distant recurrence at 10 years. However, there were no similar analyses done using patients risk-stratified by conventional criteria for comparison.

MammaPrint®

Area under the curve analysis does not suggest that MammaPrint® contributes significant risk information in addition to conventional classification criteria. In one study, after Adjuvant! Online risk classification, patients reclassified as high risk by the 70-gene signature in either Adjuvant! Online risk group had 10-year disease-free survival rates of 69%, with lower confidence limits of 45–61% and upper confidence limits of 76–84%. No published

studies retrospectively evaluated the ability of MammaPrint® to predict chemotherapy benefit in comparison to conventional criteria.

Breast Cancer Gene Expression Ratio

No ROC or reclassification analyses show whether the Breast Cancer Gene Expression Ratio better classifies conventionally classified low-risk patients according to recurrence outcomes. No published studies retrospectively evaluated the ability of the Breast Cancer Gene Expression Ratio to predict chemotherapy benefit in comparison to conventional criteria.

Discussion

Whether or not using Oncotype DX™, MammaPrint®, or the Breast Cancer Gene Expression Ratio to make management decisions regarding chemotherapy results in improved outcomes has not been tested in completely prospective randomized, controlled trials. In lieu of prospective data supporting clinical utility, retrospective data were evaluated for this Assessment.

Oncotype DX™. 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, estrogen-receptor-positive breast cancer who were treated with tamoxifen and who met other specific trial enrollment criteria (Table 7). Additionally, Oncotype DX™ provides information about the risk of recurrence that is incremental to conventional classifiers used to predict risk. Women classified as high risk by conventional methods and reclassified as low risk by Oncotype DX™ have a recurrence of at most 10–14% and likely less at lower RS values in the low-risk spectrum.

Chemotherapy provides the same proportional benefit to all patients, but the absolute benefit will be very low when the prior risk is low; such a low absolute benefit may not be perceived by patient and physician as outweighing the harms of chemotherapy. Thus, a woman who prefers to avoid the toxicity and inconvenience of chemotherapy and whose Oncotype DX™ RS value shows that she is at very low risk of recurrence might reasonably decline chemotherapy. The lower the RS value, the greater the confidence the woman can have that chemotherapy will not

provide net benefit; outcomes are improved by avoiding chemotherapy toxicity.

Recently, several organizations have updated their guidelines for recommended therapy at different levels of risk determined by Oncotype DX™:

- The ASCO “2007 Update of Recommendations for the Use of Tumor Markers in Breast Cancer” (Harris et al. 2007) indicates that the Oncotype DX™ assay can be used to predict the risk of recurrence in newly diagnosed patients with node-negative, estrogen-receptor positive breast cancer, who will be treated with tamoxifen; and to identify patients who may not require adjuvant chemotherapy. The recommendations state, “Although performed retrospectively, the validation of this assay using a prospectively collected clinical trial data set, but retrospectively collected tissues from the data set, might be considered as Level of Evidence I [best] for use of this assay.”
- The NCCN Breast Cancer Clinical Practice Guidelines (2008) indicate that the “21-gene RT-PCR assay” (Oncotype DX™) can be considered for patients with hormone receptor-positive, HER2-negative disease and tumor greater than 1 cm in size, or 0.6–1 cm and moderately/poorly differentiated or with unfavorable features, prior to making decisions about optional chemotherapy. The recommendation is categorized as “nonuniform NCCN consensus (but no major disagreement), based on lower level evidence and clinical experience.”
- The 10th St. Gallen (Switzerland) expert consensus meeting summary (Goldhirsch et al. 2007) stated that “the Panel did not accept the molecularly based tools such as Oncotype DX™... as sufficiently established to define risk categories.”

Thus, there is limited agreement that some women may benefit from using the results of the test to guide chemotherapy decisions. However, there are several limitations to the available evidence:

- Among those willing to be guided by the test result, it is unknown what proportion of conventionally estimated intermediate- to high-risk patients will have sufficiently low RS values to change their decision regarding chemotherapy.
- The recurrence risk level below which women are comfortable without

Table 7. Summary of Oncotype DX™ RS and Recurrence Risk Studies

Study	Total n	Study Objective	Results				
Study Type							
Paik et al. 2004a	668	Predict recurrence	RS Risk	% of patients		K-M Distant recurrence at 10 yr, % (95% CI)	
TAM arm of NSABP B-14 RCT			Low (<18)	51	6.8	(4.0–9.6)	
			Int (18–30)	22	14.3	(8.3–20.3)	
			High (≥31)	27	30.5	(23.6–37.4)	
			All	100	15	(12.5–17.9)	
Paik et al. 2004b	668	Reclassification study; determine incremental risk compared to conventional classifier	Risk Classification by NCCN¹	Risk Reclassification by Oncotype DX		n	% DRF at 10 yr, (95% CI)²
Additional analysis of Paik et al. 2004a data			Low (8%)	Low	38	100 (NR)	
				Int	12	80 (59–100)	
				High	3	56 (13–100)	
			High (92%)	Low	301	93 (89–96)	
				Int	137	86 (80–92)	
				High	178	70 (62–77)	
Bryant 2005	668	Reclassification study; determine incremental risk compared to conventional classifier	Risk Classification by Adjuvant! Online¹	Risk Reclassification by Oncotype DX		n	% recurrence at 10 yr, (95% CI)²
Additional analysis of Paik et al. 2004a data			Low (53%)	Low	216	5.6 (2.5–9)	
				Int-High	138	12.9 (7–19)	
			Int-High (47%)	Low	122	8.9 (4–14)	
				Int-High	192	30.7 (24–38)	
Habel et al. 2006	255 ER+ TAM+;	Predict mortality	RS Risk	10-yr Absolute Risk of Death, % (95% CI)			
Case-control	361 ER+ TAM-		Low (<18)	ER+, TAM-treated	ER+, No TAM		
			Int (18–30)	2.8 (1.7–3.9)	6.2	(4.5–7.9)	
			High (≥31)	10.7 (6.3–14.9)	17.8	(11.8–23.3)	
				15.5 (7.6–22.8)	19.9	(14.2–25.2)	

Abbreviations

DRF: distant recurrence free; ER: estrogen receptor; int: intermediate; K-M: Kaplan Meier; N: total number of patients; NCCN: National Comprehensive Cancer Network; NR: not reported; RS: Oncotype DX™ recurrence score; NSABP, National Surgical Adjuvant Breast and Bowel Project; RCT: randomized controlled trial; TAM: tamoxifen

¹ Percentages are percent of total N.

² Estimated from graphs. Note that different outcomes were reported between Paik et al. 2004b and Bryant 2005 and could not be converted to similar outcomes with confidence intervals.

chemotherapy is unknown; how the presentation of risk information affects choices is also unknown.

- Women reclassified by RS result as intermediate or high risk from conventionally estimated low risk have a much wider range of recurrence risk estimates; women with very high RS values are likely to benefit from accepting chemotherapy, but for the intermediate-risk group, benefits are uncertain.
- Because the RS is a continuous function with respect to recurrence rates, risk category cutoff values selected by the test developer are arbitrary and may not be optimal.

In addition, some patient evaluation and treatment regimens in the validation studies differ from current practices. Patients in these studies were women younger than 70 years of age (or with a life expectancy of at least 10 years) who had unilateral, non-fixed, ER-positive, node-negative (by full axillary dissection) carcinomas and who were treated with surgery (mastectomy or lumpectomy), radiation therapy, and tamoxifen. In one trial, patients in the experimental arm were also treated with CMF (cyclophosphamide, methotrexate, and 5-fluorouracil) chemotherapy. Current practices differ with respect to hormonal therapies, chemotherapy regimens, techniques for assessing lymph node status, and understanding of the role of progesterone receptor (PR) positivity.

Differences in current practices compared to the validation studies do not change our findings with respect to utility of gene expression profiling using Oncotype DX™. Aromatase inhibitor (AI)-based hormonal therapy instead of tamoxifen therapy would likely reduce recurrence rates for all RS risk groups. Thus, if a patient declined chemotherapy today on the basis of a low-risk RS (risk categories defined by outcomes after tamoxifen treatment), the even lower risk associated with AI treatment would not change that decision. For those receiving anthracycline-based chemotherapy instead of CMF, the type of chemotherapy does not change the interpretation of the Oncotype DX™ risk estimate. While current practice largely involves a detailed histologic examination of sentinel lymph nodes allowing for the detection of micrometastases (less than 2 mm in size), lymph nodes with micrometastases are not considered positive for purposes of treatment recommendations. Finally, only women with ER-positive tumors were enrolled in Oncotype DX™ validation studies, whereas

current clinical guidelines include either ER or PR positivity in the treatment pathway for women with hormone-receptor-positive early breast cancer. Recent studies show that ER-negative, PR-positive patients also tend to benefit from hormonal therapy.

Many of these limitations and differences in treatment will be addressed by the TAILORx trial, currently underway (total accrual goal ~10,000). This trial will determine disease recurrence outcomes of RS intermediate patients (RS 11–25, ~44%) in particular by randomizing ER- and/or PR-positive, lymph-node-negative (by current methods) patients with intermediate-risk RS results to hormonal therapy (either tamoxifen or AI-based) plus chemotherapy versus hormonal therapy alone. Low-risk patients (RS < 11, ~29%; treated only with hormonal therapy) will also be followed and compared to a prespecified target of no more than 5% recurrence at 10 years. The high-risk group (RS > 25, ~27%) is assumed to benefit from chemotherapy. However, the TAILORx protocol uses more conservative cutoff values to define low- and high-risk patients than used in assay validation studies, in order to help define optimal cutoff values.

The TAILORx trial is not enrolling hormone-receptor-positive, early breast cancer patients whose disease is also HER2 positive. For these patients, the current NCCN guidelines recommend a different treatment pathway once the tumor measures 1 cm or more. Published trials of trastuzumab therapy have all included concurrent chemotherapy treatment and enrolled patients with a minimum tumor size of 1 cm or more. For these patients, trial outcomes have resulted in recommendations for trastuzumab and chemotherapy in addition to hormonal therapy. Therefore, because a choice regarding chemotherapy is not indicated, these patients are not candidates for Oncotype DX™. However, as noted in the summary of the most recent St. Gallen consensus conference and resulting guideline, “The role of trastuzumab in patients with small, endocrine responsive tumors and no axillary node involvement has not been adequately evaluated.” Thus, patients with hormone receptor- and HER2-positive tumors that are smaller than 1 cm may need to decide whether to undergo chemotherapy alone and might be considered candidates for Oncotype DX™. It should be noted, however, that HER2 is represented in the Oncotype DX panel and RS results for the limited number of HER2-positive

patients in one study were all categorized as intermediate or high risk.

MammaPrint®. There is insufficient evidence to determine whether MammaPrint® is better than conventional risk assessment tools in predicting recurrence. The 10-year disease-free survival rate of patients classified as low risk was 88–89%, with lower confidence limits of 74–77%, likely too low for most patients and physicians to consider forgoing chemotherapy. One reclassification study suggests that MammaPrint® adds additional information to one conventional risk classifier; ROC analysis suggests only a small improvement with MammaPrint® classification compared to a conventional classifier. Neither ASCO, NCCN, nor St. Gallen guidelines recommend the use of MammaPrint®. A prospective, randomized trial (MINDACT) is underway to evaluate outcomes of using MammaPrint® to guide treatment.

Breast Cancer Gene Expression Ratio.

There is insufficient evidence to determine whether the Breast Cancer Gene Expression Ratio is better than conventional risk assessment tools in predicting recurrence. Recurrence rates of patients classified as low risk in available studies were 17–25%, likely too high for most patients and physicians to consider forgoing chemotherapy. There are no reclassification studies to directly compare the Breast Cancer Gene Expression Ratio with conventional risk classifiers. Neither ASCO, NCCN, nor St. Gallen guidelines recommend the use of the Breast Cancer Gene Expression Ratio.

Summary of Application of the Technology Evaluation Criteria

Based on the available evidence, the Blue Cross and Blue Shield Medical Advisory Panel made the following judgments about whether gene expression profiling for managing breast cancer treatment meets the Blue Cross and Blue Shield Association Technology Evaluation Center (TEC) criteria.

1. The technology must have final approval from the appropriate governmental regulatory bodies.

Of the three gene expression profiles commercially available in the U.S., only MammaPrint® has been cleared by the FDA (cleared February 6, 2007). MammaPrint is the first

cleared “in vitro diagnostic multivariate index assay” (IVDMIA). On September 7, 2006, the FDA issued new draft guidance for IVDMIA; subsequent to extensive public commentary, a second draft guidance was issued on July 26, 2007 (<http://www.fda.gov/cdrh/oivd/guidance/1610.pdf>). In the latter document, an IVDMIA is defined as one that “1) Combines the values of multiple variables using an interpretation function to yield a single, patient-specific result (e.g., a “classification,” “score,” “index,” etc.), that is intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment or prevention of disease, and 2) Provides a result whose derivation is non-transparent and cannot be independently derived or verified by the end user.” The comment period for the second IVDMIA draft guidance has ended, but the final disposition of the guidance is unknown at the time of this writing.

Oncotype DX™ and the Breast Cancer Gene Expression Ratio are each available from only one laboratory and are not cleared by the FDA. Clinical laboratories may develop and validate tests in-house (laboratory-developed tests or LDTs; previously called “home-brew”) and market them as a laboratory service; LDTs must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). Laboratories offering the service must be licensed by CLIA for high-complexity testing. While the FDA has technical authority to regulate LDTs, to date, there has been no active oversight, with the new exception of IVDMIA devices.

Gene expression tests that are currently being marketed for clinical use or are being used in research protocols classify patients into disease risk or treatment response categories using algorithms that incorporate the tumor expression status of multiple genes. As such, most or all of these assays are likely to fall into the IVDMIA category. Thus, it is expected that Oncotype DX™ and possibly the Breast Cancer Gene Expression Ratio would need to meet the final pre- and postmarketing device requirements once the guidance document is finalized.

In general, FDA review of laboratory tests focuses largely on technical performance, assuring the reliability of test results over time. Review of clinical performance is more limited and may be based on “existing clinical data, new clinical trial data, review of information in

the literature, or current clinical knowledge” (FDA Office of In Vitro Diagnostics 510(k) Workshop, April 19-20, 2005).

2. The scientific evidence must permit conclusions concerning the effect of the technology on health outcomes.

Oncotype DX™. The evidence is sufficient to permit conclusions regarding health outcomes. Technical performance of the assay is well documented and is unlikely to be a major source of variability; rather, tissue sampling is likely the greatest source of variability. Retrospective epidemiologic analyses indicate strong, independent associations between Oncotype DX™ RS result and distant disease recurrence or death from breast cancer. The evidence identifies a subset of conventionally classified high-risk patients who are at sufficiently low risk of recurrence by Oncotype DX™ that they might reasonably decide that the harms (toxicity) of chemotherapy outweigh the very small absolute benefit. Two studies of the original validation data, in which conventionally classified patients were reclassified by Oncotype DX™ result, indicate that the test provides significant recurrence risk information in addition to conventional criteria for individual patient risk classification. Additional evidence indicates that Oncotype DX™ results are significantly associated with breast cancer death in a community-based patient population, and that RS high-risk patients clearly benefit from chemotherapy, whereas benefits for other RS categories are not statistically significant.

MammaPrint®. There is insufficient evidence to determine whether MammaPrint® is better than conventional risk assessment tools in predicting recurrence. Limited technical performance evaluation of the commercial version of the assay suggests good reproducibility. The 10-year disease-free survival rate of patients classified as low risk was 88–89%, with lower confidence limits of 74–77%, likely too high for most patients and physicians to consider forgoing chemotherapy. One reclassification study suggests that MammaPrint® adds additional information to one conventional risk classifier; ROC analysis suggests only a small improvement with MammaPrint® classification compared to a conventional classifier.

Breast Cancer Gene Expression Ratio. There is insufficient evidence to determine whether the Breast Cancer Gene Expression Ratio is

better than conventional risk assessment tools in predicting recurrence. Assay configuration and performance characteristics of the commercially available version of the test have not been published. Recurrence rates of patients classified as low risk in available studies were 17–25%, likely too high for most patients and physicians to consider forgoing chemotherapy. There are no reclassification studies to directly compare the Breast Cancer Gene Expression Ratio with conventional risk classifiers.

3. The technology must improve the net health outcome; and

4. The technology must be as beneficial as any established alternatives.

Oncotype DX™. Oncotype DX™ gene expression profiling can improve net health outcome in women with unilateral, non-fixed, hormone receptor-positive, node-negative breast cancer. In a significant subset of cases, Oncotype DX™ is likely to change the therapy decisions a patient and her physician would otherwise make using conventional risk classifiers. Women whose Oncotype DX™ RS value shows that they are at very low risk of recurrence might reasonably choose to forgo the harms and inconvenience of chemotherapy. The lower the RS value, the greater the confidence that the woman can have that chemotherapy will not provide net benefit, thus improving outcomes. Several limitations to the available evidence indicate the need for additional study.

MammaPrint® and Breast Cancer Gene Expression Ratio. The evidence is insufficient to permit conclusions as to whether the use of MammaPrint® or the Breast Cancer Gene Expression Ratio to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy improves net health outcomes in women with early stage breast cancer.

5. The improvement must be attainable outside the investigational settings.

For Oncotype DX™, there is a single-source laboratory conducting the test; this laboratory also performed the tests for the validation studies. It is expected that the quality of diagnostic performance obtained in practice should be similar to that obtained in the published studies; however, the effect of increased demand for the test on the capacity of a single-source laboratory is unknown.

Whether MammaPrint® or the Breast Cancer Gene Expression Ratio improves the net health outcome has not been established in the investigational setting.

Based on the above, the use of Oncotype DX™ to determine recurrence risk for deciding whether or not to undergo adjuvant chemotherapy in women with unilateral, non-fixed, hormone receptor-positive, node-negative breast cancer who will receive hormonal therapy meets the TEC criteria. 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.

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Appendix I

Adverse Effects of Chemotherapy

Table A summarizes short-term adverse effects associated with different adjuvant chemotherapy regimens for breast cancer. Long-term adverse effects include premature menopause, a common consequence of most chemotherapy regimens (Table B). Although ovarian failure may be beneficial in terms of disease, women who experience premature menopause have accelerated bone mineral density loss as well as the common symptoms of menopause. Although some women regain menstrual function after treatment, those who remain amenorrheic 1 year after treatment seldom regain function (Partridge et al. 2001).

Weight gain in conjunction with adjuvant chemotherapy has been reported in 50% or more of patients, and is more common in premenopausal women, women who experience menopause as a result of chemotherapy, and in women administered longer treatment regimens. Substantial weight gain can have negative consequences for physical and psychological health.

Cardiotoxicity is a major concern when anthracycline-based regimens are used, particularly in women who are older and in those with a history of cardiac disease. Congestive heart failure has been reported in approximately 1–2% of patients, and systolic dysfunction (ejection fraction of less than 55%) in 8% (Partridge et al. 2001). Aggregate analysis of trials comparing an anthracycline to no or to CMF chemotherapy shows a trend for increased death from heart disease but no statistically significant difference, though hazards may prove to be significant with increased follow-up

or certain anthracycline regimens (EBCTCG 2005). The potential for cardiotoxicity may be increased by as much as 4% in women treated with an anthracycline who are also administered trastuzumab (Carlson et al. 2006).

Leukemia or myelodysplastic syndromes are serious adjuvant chemotherapy complications to be considered when the risk of breast cancer recurrence is low. The incidence of leukemia for anthracycline-based regimens is approximately 0.1–1.5% over 5–10 years of follow-up (Partridge et al. 2001) and for non-anthracycline-containing regimens is estimated at 5 cases in 10,000 women over 10 years (Curtis et al. 1992).

Evidence suggests that systemic adjuvant chemotherapy may result in cognitive dysfunction with a significant impact on quality of life in at least a subset of patients (Tannock et al. 2004; Rugo and Ahles 2003). However, while completed studies consistently show an effect, limitations in study design suggest a need for well-planned, confirmatory studies (Phillips and Bernhard 2003).

More recently, docetaxel, used frequently in the treatment of metastatic breast cancer, is now often used in place of paclitaxel as part of combination therapy in the adjuvant and neoadjuvant settings in early stage breast cancer (NCCN 2008). Adverse effects include myelosuppression, neutropenia, diarrhea, peripheral neuropathy, edema, alopecia, and, rarely, severe hypersensitivity reactions (Hainsworth 2004).

Table A. Frequency and Usual Severity¹ of Short-term Adverse Effects by Regimen²
(Reprinted from Partridge et al. *J Natl Cancer Inst Monogr*, 2001; 30:135-42 with permission of the National Cancer Society.)

Chemotherapy Regimen	Nausea	Vomiting	Diarrhea	Stomatitis	Alopecia	Neutropenia	Febrile Neutropenia or Infection	Thrombocytopenia	Neuropathy	Myalgias
CMF (oral C)	Frequent, +/+++	Common, +	Common, +	Common, +	Frequent, partial-total	Frequent, ++/+++	Rare	Frequent, +	Almost never [†]	Almost never [†]
CMF (all IV)	Common, +/++	Frequent, +	Common, +	Uncommon, +	Frequent, partial-total	Frequent, ++/+++	Rare	Uncommon, +	Almost never [†]	Almost never [†]
MF	Common, +	Common, +	Common, +/+++	Uncommon, +	Uncommon, minimal	Rare, +	Almost never	Almost never [†]	Almost never [†]	Almost never [†]
AC	Frequent, +/+++	Common, +/++	Uncommon, +	Common, +/++	Almost always, total	Frequent, ++/+++	Rare	Uncommon, +	Almost never [†]	Almost never [†]
AC-paclitaxel (paclitaxel only)	Rare, +	Rare, +	Rare, +	Rare, +	Almost always, total	Common, +	Rare	Almost never [†]	Uncommon, +/++	Common, +/++
CEF/FAC (oral C)	Frequent, ++/+++	Frequent, +/++	Common, +/+++	Frequent, ++/+++	Almost always, total	Almost always, +++	Common	Frequent, +/++	Uncommon, +	Uncommon, +
CAF/FAC/FEC 100 (all IV)	Common, ++/+++	Common, +/++	Common, +/+++	Frequent, +/++	Almost always, total	Frequent, +++	Common	Frequent, +/++	Uncommon, +	Uncommon, +

¹ The indicated rates of nausea and vomiting may be higher than currently experienced, as some trials were conducted before the availability of newer antiemetic agents.

² Frequency: almost never = less than 1%; rare = 1-5%; uncommon = 6-20%; common = 21-50%; frequent = 51-95%; almost always = more than 95%. Severity (for all toxic effects excluding alopecia): + = mild; ++ = moderate; +++ = severe. CMF: cyclophosphamide, methotrexate, and 5-fluorouracil; AC: doxorubicin and cyclophosphamide; CAF: cyclophosphamide, doxorubicin, and 5-fluorouracil; FEC: 5-fluorouracil, epirubicin, and cyclophosphamide; MF: methotrexate and 5-fluorouracil; CEF: cyclophosphamide, epirubicin, and 5-fluorouracil; FAC: 5-fluorouracil, doxorubicin, and cyclophosphamide.

[†] Not recorded.

Table B. Risk of Premature Menopause by Regimen and Age*
 (Reprinted from Partridge et al. *J Natl Cancer Inst Monogr*, 2001; 30:135-42 with permission of the National Cancer Society.)

Regimen	Duration, mos.	Incidence of amenorrhea, %	
		<40 yr	≥40 yr
CMF-based	6	31–38	81–92
	12	51–77	83–98
FEC	6	23	89
AC	3	13	57–63
MF	6	~10	

*Adapted with permission from Burstein and Winer in J.R. Harris' *Diseases of the Breast*, 2000.

CMF = cyclophosphamide, methotrexate, and 5-fluorouracil; FEC = 5-fluorouracil, epirubicin, and cyclophosphamide; AC = doxorubicin and cyclophosphamide; MF = methotrexate and 5-fluorouracil.

Appendix II

Gene Expression Technology

As noted in the text, broad-scope genomic DNA microarrays can be used to search for specific genetic markers with strong associations with an outcome of interest, e.g., cancer recurrence, using tumor tissue samples from clinically representative populations. Another option is to select a large number of candidate genes already known to be associated with the cancer or process of interest, and using representative samples identify those with the strongest predictive value. Both approaches have advantages and disadvantages (Table C).

Microarrays consist of numerous DNA sequences deposited in microscopic quantities at ordered locations on a solid surface, like a glass slide. DNA prepared from tumor tissue samples can be applied to the surface of the microarray; any complementary sequences bind to the microarray sequences. Excess sample is washed away and any bound sample DNA is detected. Various types of complex

analysis methods can be used to determine which detected markers best correlate with the outcome of interest. Analysis methods are hotly debated and often misapplied (Dupuy and Simon 2007). Once chosen, microarray technology can continue to be used as the test format, using only information from the selected markers, or a simpler technology such as reverse transcriptase-polymerase chain reaction (RT-PCR) can be used.

To measure gene expression using RT-PCR, messenger RNA (mRNA) extracted from a sample of interest is reverse-transcribed into cDNA, which is then quantitatively amplified using PCR. High accuracy and exceptional sensitivity can be achieved but the method requires detailed knowledge of the DNA sequence of interest. Thus, RT-PCR can be used when a smaller number of well-defined markers have already been characterized and selected.

Table C. Methods of Developing Gene Expression Panels

Approach	Advantages	Limitations
A. Candidate genes chosen a priori from known gene-tumor associations; may be developed into microarray assays using related sequences	<ul style="list-style-type: none"> – Uses existing knowledge about specific genes and gene products in relation to the cancer of interest – Hypothesis-driven – Fastest approach – Least expensive 	<p>Only helpful if:</p> <ul style="list-style-type: none"> – Strong association of altered expression with tumor behavior or characteristic – A priori choices and hypotheses are correct
B. Genomics pattern analysis e.g., from broad scope cDNA microarrays	<ul style="list-style-type: none"> – No a priori hypothesis or choice of markers – Based on broad scope or complete genome expression profile – May identify previously unsuspected associations – Provides a large amount of data 	<ul style="list-style-type: none"> – Does not use information on known biological associations – May provide too much data for convenient analysis – Optimal data management and analytic methods under debate – Likely slower than A. – Expensive – ? practical in a clinical setting

Appendix III

Gene Expression Panels Not Yet Commercially Available in the U.S.

Table D. Gene Expression Panels in Development for Predicting Breast Cancer Recurrence and Benefit from Adjuvant Chemotherapy

Panel Name (source)	Target Population	Panel Description	Specimen	Validation: Recurrence Prediction	Results Summary
76-gene signature (Veridex—Johnson & Johnson)	Not age-limited. Node-negative patients, no adjuvant systemic treatment; mostly T1-2 or tumor size <5 cm	76 cancer-related genes and 221 control genes selected using cDNA microarray analysis (22,000 transcripts) of tumor gene expression (Wang et al. 2005).	Frozen tumor specimens	n=115 Training set n=171 Test set n=180 Multicenter study	<p>Multivariable analysis of test set, controlling for traditional clinical predictors of risk: 5-yr distant metastasis-free survival hazard ratio for 76-gene signature = 5.6 (95% CI: 2.5-12). Results independent of ER status. 52 of 60 distant relapses correctly predicted. 52% of patients recommended for adjuvant chemotherapy by 76-gene signature, 90% by St. Gallen and 89% by NIH guidelines. (Wang et al. 2005)</p> <p>Multicenter study: Multivariable (as above) 5-yr distant metastasis-free survival hazard ratio 11.4 (95% CI: 2.7–48). 27 of 30 distant relapses correctly predicted. 43% of clinically low-risk patients recommended for adjuvant chemotherapy by 76-gene signature, 95% by St. Gallen and 98% by NIH guidelines. (Foekens et al. 2006)</p> <p>In progress: evaluating a cohort of lymph-node-negative breast cancer patients treated with tamoxifen.</p>
Ahr et al. Goethe University	Lymph node status N0-1	41 gene marker panel selected using cDNA microarray analysis (588 and 45,000 transcripts) of tumor gene expression (Ahr et al. 2001)	?Fresh tissue	n=17 Training set n=55 Test set	<p>Test set: 11 of the 22 patients in the high risk subgroup progressed to metastatic disease within a median of 23.5 months. Only 3 of 27 cases who were not classified high risk by gene expression developed metastatic disease (p=0.016). The association was independent of nodal status. (Ahr et al. 2002)</p>

Table D. Gene Expression Panels in Development for Predicting Breast Cancer Recurrence and Benefit from Adjuvant Chemotherapy (cont'd)

Panel Name (source)	Target Population	Panel Description	Specimen	Validation: Recurrence Prediction	Results Summary
Intrinsic subtypes	Breast carcinoma; one subset (n=51) locally advanced, treated with neoadjuvant chemotherapy, surgery, and TAM	Intrinsic gene set selected from 8,102 genes to optimally identify the intrinsic characteristics of breast tumors; intrinsic gene set composed of 456, 264, 534, 93, or 1,300 genes, depending on the publication	Snap-frozen tumor specimens	<p>n=65 (45 pts) n=78 (77 pts; subset: n=51) Training sets</p> <p>n=99, 115 Test sets</p> <p>n=105 New training set</p> <p>n=311, 60 Test sets</p>	<p>Hierarchical clustering of expression results resulted in classification into 5 subtypes: basal-like, ERBB2+, normal breast-like, luminal A, and luminal B (Perou et al. 2000; Sorlie et al. 2001). For 49 of 51 patients with locally advanced disease, basal-like and ERBB2+ subtypes were associated with the shortest relapse-free and overall survival times (p<0.01; Sorlie et al. 2001).</p> <p>99 unselected, LN+/- tumor specimens were classified by hierarchical clustering into basal-like and luminal subgroups; 16 genes were significantly associated with relapse-free survival after accounting for multiple comparisons (Sotiriou et al. 2003).</p> <p>115 tumors were classified into 5 subtypes; similar subtypes were found by applying cluster analysis to two published, independent data sets (Sorlie et al. 2003).</p> <p>A new intrinsic list of 1,300 genes was used to classify (into 5 subtypes) and predict survival on a data set of 311 tumors from 3 different microarray studies. These classifications added prognostic information independent of traditional clinical predictors. An "objective and unchanging classifier" was derived from the results for used in predicting prognosis from single samples. This final predictor was significantly associated with recurrence-free and overall survival in 2 separate datasets (Hu et al. 2006).</p>

Table D. Gene Expression Panels in Development for Predicting Breast Cancer Recurrence and Benefit from Adjuvant Chemotherapy (cont'd)

Panel Name (source)	Target Population	Panel Description	Specimen	Validation: Recurrence Prediction	Results Summary
Wound-response signature	Applied to basal-luminal test set database and MammaPrint® training and test set databases	Expression of 512 genes selected for cultured fibroblast response to serum, omitting genes directly related to cell proliferation (Chang et al. 2004)	Fresh frozen tumor tissue	n=50 Training set n=51, 78, 295 Test sets	<p>Test sets:</p> <p>In a set of 51 patients with locally advanced breast cancer, treated with neoadjuvant chemotherapy and adjuvant TAM, the wound-response signature was associated with a greater likelihood of metastasis (p=0.013) and death (p=0.041) after a 5-yr follow-up (Chang et al. 2004).</p> <p>In 78 patients with LN- tumors ≤5 cm in diameter and who had not been treated with tamoxifen, those with the wound-response signature had a significantly increased risk of metastasis over 5 yr (p=0.00046; Chang et al. 2004).</p> <p>In a set of 295 stage I or II breast cancer patients, most treated with chemotherapy and/or TAM, presence of the wound-response signature was significantly associated with distant metastasis (p=8.6×10⁻⁶) and death (p=5.6×10⁻¹⁰). The signature was an independent predictor and provided more information than classic risk factors. Use of the signature would have spared 30% of the women who did not develop metastasis from exposure to chemotherapy. The 70-gene and wound-response signatures were sufficient to capture most of the prognostic information (Chang et al. 2005).</p>

Table E. Single Publications Describing a New Gene Expression Analysis of Early Breast Cancer

First Author	Year	Predictor
Huang E	2003	Metagenes (aggregate patterns of gene expression) that associate with lymph node status and recurrence in heterogeneous tumor samples
Shen R	2004	90-gene "meta-signature" strongly associated with survival in heterogeneous tumor samples
Pawitan Y	2005	64 genes associated with distant metastasis or death in heterogeneous tumor samples
Tsumagari K	2005	58 genes associated with disease-free survival in lymph node-negative breast cancer patients
Jansen MP	2005	44 gene signature predicting tamoxifen response in ER+ patients
Linke SP	2006	BCL2, ERBB2, MYC, and TP53 add value to traditional clinical parameters in predicting recurrence-free and overall survival in tamoxifen-treated breast cancer patients
Oh DS	2006	822 genes optimally predicted 2 groups with good vs. poor survival in ER+ and/or PR+ patients
Cheng SH	2006	Gene expression prediction model for locoregional recurrence, to select patients for post-mastectomy radiotherapy
Sotiriou C	2006	Identify and evaluate gene expression profiles to improve histologic grading



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