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# Emerging Data on Multianalyte Algorithm Assays in Breast Cancer: A Clinical Review

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# Translating Research into Practice: the Prosigna® (PAM50) Gene Signature Assay

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**Abstract:** Gene expression analyses using DNA microarrays found that breast cancer tumors can be classified into 4 main subtypes: Luminal A, Luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, and basal-like. These intrinsic subtypes differ in their relapse patterns. For example, Luminal A breast cancer is associated with a low risk of relapse, but the time frame for relapse can extend beyond 10 years. In basal-type disease, relapses typically occur within the first 5 years. The prediction analysis of microarray PAM50 gene set is the standard test used for identifying the gene expression–based intrinsic subtypes in breast cancer. Studies suggest that the PAM50 gene set assay can be used to help predict prognosis in metastatic breast cancer, risk of recurrence in estrogen receptor–positive patients, and benefit of chemotherapy. Multiple laboratory techniques can be used to quantify gene expression, including the nCounter system, which can be used to evaluate expression of multiple genes simultaneously and does not require signal amplification for detection. In the future, gene signatures may allow selection of specific chemotherapy agents for certain patients.

Gene expression analyses have identified molecular signatures that reflect biological differences among breast cancers. These signatures can help predict outcome and, in some cases, the potential benefit of adjuvant therapy. Several multigene assays are used in the management of patients with early breast cancer. The prediction analysis of microarray PAM50 gene set is the standard for identifying the gene expression–based "intrinsic" subtypes in breast cancer. The intrinsic subtypes are defined based on specific gene expression patterns that reflect differences in tumor biology.

## Evolution of Intrinsic Subtype in Breast Cancer

### *Identification of Breast Cancer Subtypes*

The development of the PAM50 signature began more than 15 years ago with the identification of specific patterns of gene expression in patients with early-stage breast cancer.[1] Gene expression analyses using DNA microarrays found that tumors could be classified into 4 main subtypes: Luminal A, Luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, and basal-like.[1] Since the initial description of the intrinsic subtypes, multiple studies have validated their characteristics and prognostic significance.[2-4]

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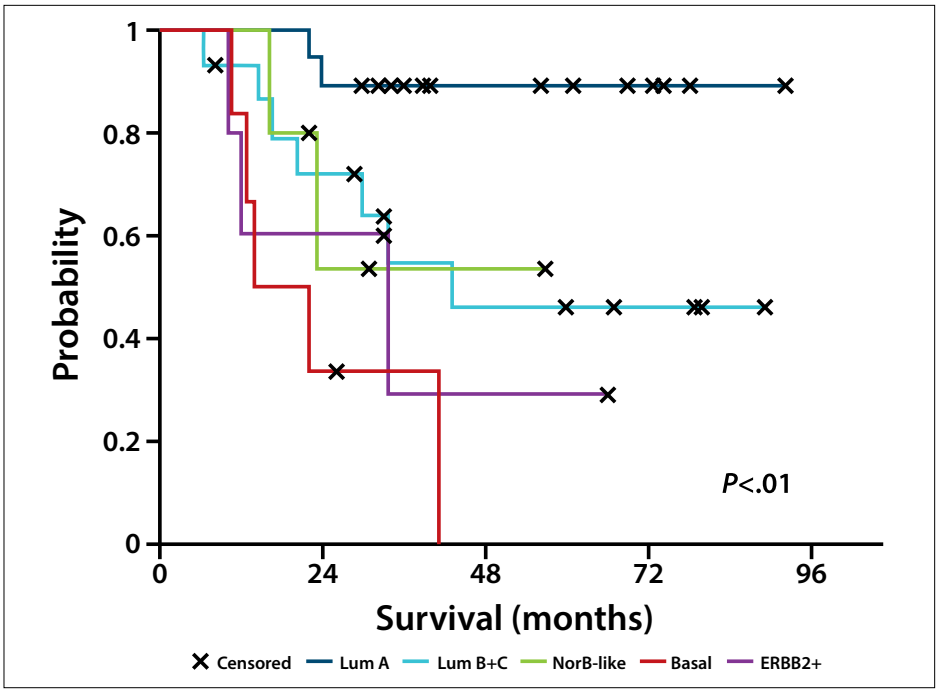


**Figure 1.** Intrinsic subtypes differ in their relapse patterns. Adapted from Sørlie T et al. *Proc Natl Acad Sci U S A*. 2001;98(19):10869-10874.[2]

The vast majority of luminal-type breast cancers are estrogen receptor (ER)–positive tumors.[5] Luminal A is associated with a favorable prognosis and demonstrates high response rates to endocrine therapy alone. Luminal B is associated with a higher risk of relapse with endocrine therapy alone. The majority of basal-like tumors are triple-negative, lacking expression of the ER, the progesterone receptor (PR), and HER2.[6] Patients with HER2-enriched subtypes that express *ERBB2* have been shown to have the worst prognosis in the absence of adjuvant anti-HER treatment.[7]

Intrinsic subtypes differ in their relapse patterns (Figure 1).[2] Although Luminal A breast cancer is associated with a low risk of relapse, the time frame in which patients are at risk for relapse tends to be long, with some relapses occurring after 10 years. In contrast, in basal-type disease, relapses typically occur within the first 5 years.

### *Development of a Clinical Test to Diagnose Intrinsic Subtypes*

The prognostic significance of the intrinsic subtypes led to the development of a clinical test for identification in individual patients. Parker and colleagues evaluated 189 prototype breast cancer samples for expression patterns of 1906 "intrinsic" genes.[7] After identifying 122 breast cancers with significant clusters representing the range of intrinsic subtypes, quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) data were used to reduce the gene set to 50 genes that could accurately distinguish the intrinsic subtypes.

Cross-validation studies confirmed the robustness of the gene set, with 94% concordance to the full intrinsic gene set.[7] The 50-gene set was evaluated for reproducibility using several centroid-based prediction models, in which expression of each gene is compared against the average expression in a prototype sample, and the subtype classification is assigned to the centroid with the greatest correlation.[8,9] The PAM algorithm yielded accurate and reproducible subtype classifications and was selected as a model for predicting intrinsic subtype in test samples.[7] This gene set, referred to as the PAM50 classifier, has become the standard for determining intrinsic subtypes.

The analysis by Parker and colleagues found that the association between high-risk scores and a higher probability of pathologic complete response supports the conclusion that indolent ER-positive tumors (Luminal A) are less responsive to chemotherapy.[7] Parker and colleagues detected a plateau for the risk of relapse vs the probability of pathologic complete response (unlike risk of relapse for prognosis), confirming the presence of significant chemotherapy resistance among tumors at highest risk.[7]

Bastien and colleagues evaluated the concordance of biomarker expression (ER, PR, and HER2) as assessed by PAM50 qRT-PCR vs standard immunohistochemistry (IHC).[9] A training set was developed based on 171 breast samples, including 155 breast cancers and 16 normal tissue samples. Analyses were then conducted on 814 test samples derived from patients with locally advanced primary invasive breast cancer enrolled in the Grupo Español

**Table 1.** Histologic Scoring Across PAM50 Subtypes

| | Grade | ER | PR | *HER2* |
|---|---|---|---|---|
| Luminal A n=277 | G1-68 (25%) | Neg-19 (7%) | Neg-16 (6%) | Neg-273 (99%) |
| | G2-142 (51%) | Pos-258 (93%) | Pos-261 (94%) | Pos-4 (1%) |
| | G3-39 (14%) | | | |
| | GX-28 (10%) | | | |
| Luminal B n=261 | G1-25 (10%) | Neg-22 (8%) | Neg-68 (26%) | Neg-224 (86%) |
| | G2-111 (43%) | Pos-239 (92%) | Pos-193 (74%) | Pos-37 (14%) |
| | G3-111 (43%) | | | |
| | GX-14 (5%) | | | |
| HER2-enriched n=174 | G1-6 (3%) | Neg-63 (36%) | Neg-93 (53%) | Neg-105 (60%) |
| | G2-65 (37%) | Pos-111 (64%) | Pos-81 (47%) | Pos-69 (40%) |
| | G3-96 (55%) | | | |
| | GX-7 (4%) | | | |
| Basal n=70 | G1-0 (0%) | Neg-63 (90%) | Neg-62 (89%) | Neg-67 (96%) |
| | G2-4 (6%) | Pos-7 (10%) | Pos-8 (11%) | Pos-3 (4%) |
| | G3-61 (87%) | | | |
| | GX-5 (7%) | | | |

ER, estrogen-receptor; *HER2*, human epidermal growth factor receptor 2; Neg, negative; Pos, positive; PR, progesterone receptor.

Data from Bastien RR et al. *BMC Med Genomics*. 2012;5:44.[9]

de Investigación en Cáncer de Mama (GEICAM)/9906 phase 3 clinical trial.[9] Expression of *ESR1, PGR,* and *ERBB2* showed high agreement with established IHC cutoffs. Of the samples testing luminal type (A or B) by PAM50, 92% were ER-positive by IHC (Table 1). However, only 75% of ER-negative tumors were HER2-enriched or basal-like. Among the tumors testing ER-negative and HER2-positive by IHC, 77% were classified as HER2-enriched. Among the triple-negative samples (negative for ER, PR, and HER2), 57% were found to be basal-like and 30% were HER2-enriched. These discrepancies between the IHC profile and the intrinsic subtype suggest that a standard IHC panel of ER, PR, and HER2 does not sufficiently identify the intrinsic subtypes. Moreover, in a multivariate analysis, single-gene scoring for *ESR1*, *PGR*, and *ERBB2* expression derived from the PAM50 qRT-PCR was more prognostic than the corresponding IHC markers.[9]

### *Recognition of Significance of Intrinsic Subtype*

A comprehensive analysis by the Cancer Genome Atlas Network confirmed the importance of the intrinsic subtype, demonstrating that the spectrum of genetic and epigenetic abnormalities found in breast cancer tumors can be generally grouped into the 4 main subtypes.[6] Recently updated guidelines from the German Association of Gynecological Oncology have included the Prosigna® Assay for use in newly diagnosed patients with node-negative or node-positive, HR-positive, HER2-negative, early-stage breast cancer who lack clinicopathologic characteristics that indicate a clear therapeutic decision.[10] The 2013 St Gallen breast cancer treatment recommendations, drafted by an independent academic expert panel, also recognized the value of identifying the breast cancer subtype.[11] The panel noted that the intrinsic subtype, including those defined by clinicopathologic surrogates (ER, PR, HER2 expression, and Ki-67 activity) should influence the decision of whether to use adjuvant chemotherapy.[11] The breast cancer guidelines from the National Comprehensive Cancer Network mention 5 major subtypes identified by DNA microarray gene expression profiling: ER-positive/HER2-negative (Luminal A and Luminal B subtypes); ER-negative/HER2-negative (basal subtype); HER2-positive; and tumors that are similar to normal breast tissue. The guidelines state that these gene expression subtypes have been associated with differing relapse-free survival and overall survival in retrospective analyses.[12]

The role of molecular subtyping in clinical practice may differ based on the phenotypic information already available. For example, if HER2 positivity has already been identified, the standard therapy is trastuzumab administered with chemotherapy, regardless of the intrinsic subtype.[12] Similarly, for patients with basal-type, triple-negative tumors, chemotherapy is generally required, and endocrine therapy would not be appropriate.[12] In these cases, identifying the intrinsic subtype

would not change the treatment decision. Data suggest that HER2-positive and HER2-enriched patients derive the greatest benefit from anti-HER2 therapy.[13] Patients with triple-negative breast cancer may benefit from one class of chemotherapy vs another.[14] Although the available data do not yet warrant a change in clinical practice, they suggest that in the future, gene signatures may allow selection of specific chemotherapy agents for certain patients.

### *Methods for Determining Intrinsic Subtype*

Although the PAM50 gene set is considered to be the gold standard molecular assay for determining intrinsic subtype, the BluePrint® assay is another option. It assesses breast cancer subtype using an 80-gene assay and, in contrast to PAM50, was developed in a supervised manner to predict subtypes defined by IHC markers. BluePrint functional molecular subtyping classifies breast cancer into luminal-type, HER2-type, and basal-type disease.[15]

## Evidence for Clinical Utility of Research into the PAM50 Signature

Following the identification of the PAM50 gene signature, research studies were undertaken to evaluate the potential clinical utility of the signature, including its utility for estimating prognosis in ER-positive, early-stage breast cancer and metastatic breast cancer and for predicting benefit from chemotherapy (anthracyclines, taxanes, and gemcitabine) and HER2-targeted therapy.

### *Predicting Risk of Recurrence in ER-Positive Patients*

After developing the PAM50 gene set, Parker and colleagues confirmed the significance of the intrinsic subtype identified by PAM50 in both untreated patients and in those receiving neoadjuvant chemotherapy.[7] The intrinsic subtype showed significant prognostic value ($P$=2.2 × $10^{-12}$) that retained significance in a multivariate analysis accounting for ER status, histologic grade, tumor size, and nodal status.[7]

Another study evaluated the prognostic value of the PAM50 gene expression signature in patients who had received tamoxifen.[16] Nielsen and colleagues analyzed the PAM50 gene set on 786 formalin-fixed paraffin-embedded tumor specimens from an independent cohort of patients with invasive breast cancer. At a median follow-up of 11.7 years, the PAM50 signature was found to yield more prognostic information than the clinical assays for hormone receptors or Ki-67 activity.[16]

Chia and colleagues evaluated data from the National Institute of Canada Clinical Trials Group MA.12 study, a prospective, randomized trial comparing cyclophosphamide, methotrexate, and fluorouracil vs cyclophosphamide, epirubicin, and fluorouracil as adjuvant therapy in premenopausal women with node-positive breast cancer, to determine the prognostic and predictive significance of intrinsic subtypes identified by the PAM50 gene set and by IHC.[17] Classification by the PAM50 Assay was significantly prognostic for disease-free survival ($P$=.0003) and overall survival ($P$=.0002), whereas classification by IHC was not. PAM50 was prognostic in premenopausal patients treated with chemotherapy and tamoxifen. Luminal subtypes also predicted whether a patient would benefit from tamoxifen. Immunohistochemistry subtyping and ER or PR status were not predictive of a benefit with tamoxifen.

### *Predicting Prognosis in Metastatic Breast Cancer*

The prognostic value of the PAM50 signature has also been evaluated in patients with metastatic breast cancer. In an analysis of formalin-fixed, paraffin-embedded primary breast tumor samples from 270 patients with advanced breast cancer, the intrinsic subtype as determined by PAM50 was a significant independent prognostic factor for time-to-progression ($P$=.014) and overall survival ($P$=.0003).[18]

### *Predicting Benefit of Chemotherapy*

Analyses have been conducted to evaluate the potential use of the PAM50 Assay to predict responses to chemotherapy. Small studies and retrospective analyses have reported that intrinsic subtypes differ in their response to various chemotherapeutic agents.[19-21] Based on these reports, larger analyses were initiated. Cheang and colleagues[22] evaluated the utility of the PAM50 gene set to predict responses to anthracycline-based chemotherapy using 476 tumor specimens from the National Cancer Institute of Canada Clinical Trials Group MA.5 trial.[23] In a combined analysis of both arms (cyclophosphamide, methotrexate, and fluorouracil vs cyclophosphamide, epirubicin, and fluorouracil), the intrinsic subtype was significantly associated with relapse-free survival ($P$=.0005) and overall survival ($P$<.0001).[22] In an analysis of treatment effect, the HER2-enriched subtype was strongly associated with a greater benefit from anthracycline-based chemotherapy; the difference between arms in 5-year relapse-free survival and overall survival exceeded 20% in the HER2-enriched subtype, compared with less than 2% in other subtypes (overall survival interaction, $P$=.0008).[22] In patients with basal-like tumors, there was no difference in outcomes between the arms, suggesting no benefit with anthracyclines in these patients with tumors typically regarded as chemotherapy-sensitive.

Martín and colleagues evaluated whether the PAM50 intrinsic subtype classification and the 11-gene proliferation score contained within the PAM50 Assay

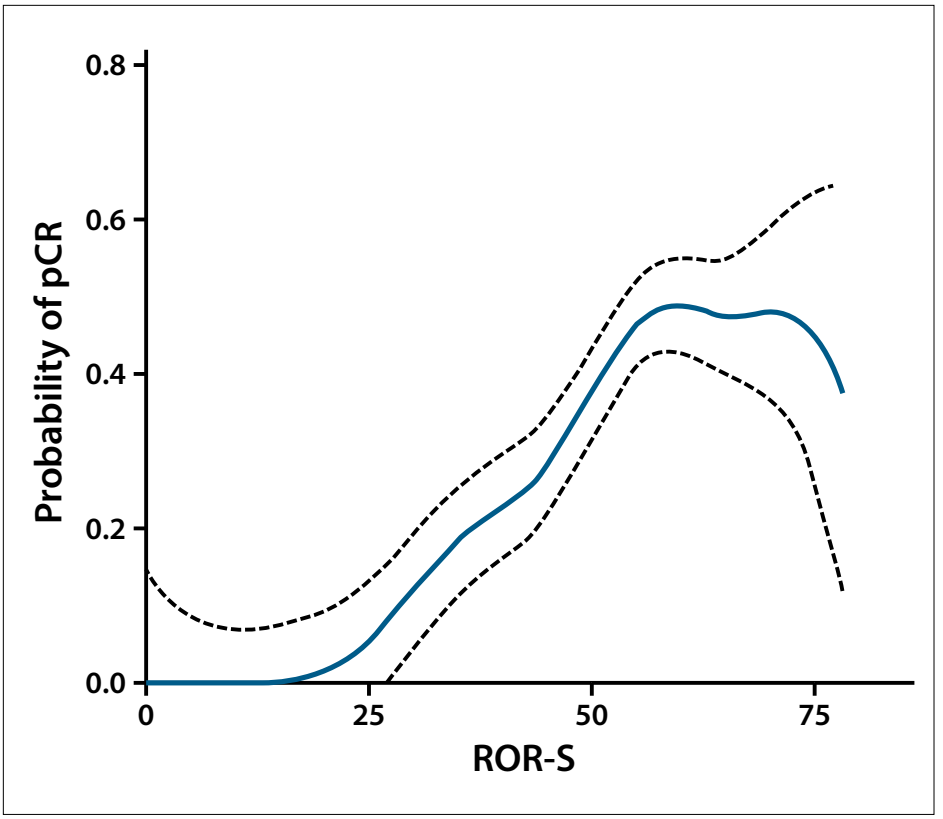


**Figure 2.** ROR-S vs probability of pCR among patients receiving neoadjuvant paclitaxel, fluorouracil, doxorubicin, and cyclophosphamide. pCR, pathologic complete response; ROR-S, risk of relapse score. Adapted from Parker JS et al. *J Clin Oncol.* 2009;27:1160-1167.[7]

could predict benefit among patients with node-positive breast cancer who received weekly paclitaxel in addition to anthracycline-containing adjuvant chemotherapy.[24] The researchers used tissue samples from the phase 3 GEICAM/9906 trial,[25] which compared cyclophosphamide/epirubicin/fluorouracil alone or followed by weekly paclitaxel as adjuvant therapy. The PAM50 analysis found that only the subset of patients with a low PAM50 proliferation score had a benefit from paclitaxel (unadjusted hazard ratio [HR], 0.23; *P*<.001; interaction test, *P*=.006). A similar analysis of patients enrolled in the 9342[26] and 9840[27] trials from the Cancer and Leukemia Group B found a benefit from weekly paclitaxel in only those patients whose tumors had a low PAM50 proliferation score (3 times weekly vs weekly, unadjusted HR, 2.09 within the lowest quartile; CI, 1.17-3.32; *P*=.0057). A formal test of interaction between the PAM50 proliferation score and treatment did not reach statistical significance (*P*=.109). The individual PAM50 subtypes did not predict efficacy of weekly paclitaxel.[24]

The utility of the PAM50 gene set and proliferation signature was evaluated in patients with clinically or molecularly determined triple-negative breast cancer or basal-like breast cancer by Prat and colleagues.[13] In an analysis of 1055 patients with triple-negative breast cancer, basal-like cancer, or both, the PAM50 proliferation signature was significantly predictive of responses to chemotherapy and extended survival after chemotherapy only in patients with the basal-like intrinsic subtype.[13]

Prat and coworkers also performed a retrospective exploratory analysis of the NOAH (Neoadjuvant Herceptin) trial of women with HER2-positive, locally advanced or inflammatory breast cancer treated with neoadjuvant chemotherapy.[28] The study found that trastuzumab-based chemotherapy was especially beneficial in women with advanced HER2-positive/HER2-enriched tumors and HER2-positive tumors that were predicted to have a high risk of relapse and proliferation status as identified by the PAM50 Assay. A stronger trastuzumab benefit was observed in the HER2-enriched tumors (event-free survival HR, 0.430) compared with non–HER2-enriched tumors (event-free survival HR, 0.807). Within hormone receptor–negative disease, patients with HER2-enriched tumors showed a better outcome than those with tumors that were not enriched for HER2.

In their analysis of the utility of the PAM50 intrinsic subtype in patients with advanced cancer, Jørgensen and colleagues also evaluated the ability of the intrinsic

subtype to predict responses to chemotherapy.[18] Although the PAM50 intrinsic subtype did not predict differential response rates between the arms, it did significantly predict overall survival following gemcitabine plus docetaxel vs docetaxel alone ($P$=.0016 for interaction).

In a study by Tutt and colleagues, patients with *BRCA1/2* mutations had a stronger response and a longer progression-free survival with carboplatin than patients without the mutation.[14] This trial compared treatment with carboplatin vs docetaxel in 376 patients, most of whom (91%) had metastatic disease. Among the 43 patients with the *BRCA* mutation, the objective response rate was 68% with carboplatin and 33% with docetaxel, a 34.7% absolute difference (95% CI, 6.3%-63.1%; $P$=.03). The *BRCA*-negative patients showed no significant difference for either treatment. This study also found that among patients with triple-negative breast cancer, those with the non-basal subtype—as identified by the PAM50 Assay—were more likely to respond to docetaxel than carboplatin (73.7% vs 16.7%; $P$<.01). Parker and colleagues demonstrated that response to chemotherapy (improvement of pathologic complete response) corresponded to an increase in the risk of recurrence (ROR) score provided by the PAM50 Assay (Figure 2).[7]

Smaller studies are evaluating the predictive value of the PAM50 intrinsic subtype in other therapeutic settings and assessing the role of gene signatures to help clinicians manage breast cancer throughout the disease process.

### *Transition to a Clinical Assay*

To transition the PAM50 gene signature from research to clinical practice, the PAM50 algorithm was re-optimized to quantify the ROR on the NanoString nCounter® Dx Analysis System. To generate the algorithm, normalized 50-gene prototypical gene expression profiles (centroids) were developed for the 4 intrinsic subtypes based on a retrospective set of more than 500 formalin-fixed, paraffin-embedded tumor samples from North America (the training set).[7] Using a computational algorithm based on a Pearson's correlation, gene expression patterns from a normalized test sample were compared against the PAM50 centroids. A Cox model was then constructed using the Pearson correlation of a 46-gene subset to each PAM50 centroid, a proliferation score, and the gross tumor size. This model generated the Prosigna Score (also called the ROR score), which is adjusted to a scale ranging from 0 to 100.[7] In a validation study by Dowsett and colleagues that evaluated tumor samples from 1017 patients who received anastrozole or tamoxifen in the ATAC (Arimidex, Tamoxifen Alone or Combined) trial, correlation between the 46-gene and 50-gene signature was high (0.998), and results between the 2 gene sets were almost identical irrespective of tumor size inclusion.[29]

The Prosigna Score and nodal status are used to assign patients to a risk category (low, intermediate, or high) that reflects the probability of distant recurrence at 10 years, for postmenopausal women with hormone receptor–positive, early-stage breast cancer.[30]

### *Switching to the nCounter System*

Multiple laboratory techniques can be used to quantify gene expression. Microarray analysis and qRT-PCR have been used to assess the prognostic value of the PAM50 Assay. The nCounter system is an alternative platform for evaluating expression of multiple genes simultaneously.[30] The technology differs from other methods in that it does not require signal amplification for detection. Instead, the system involves direct hybridization of the RNA to fluorescent reporter and capture probes that are unique for each target molecule of interest.[30] The nCounter tool uses only a small amount of tissue, and it is adaptable, enabling the interrogation of any tissue for gene expression patterns, DNA copy number aberrations, or microRNA expression patterns.

There are several other benefits to the nCounter system. The assay uses a relatively simple process that requires no enzymes, reducing the bias associated with enzymatic reactions. Moreover, the system can be decentralized and located in local laboratories or clinics, similar to the way a complete blood count is assessed. The nCounter assay also provides results more quickly than other gene expression assays. The assay requires 3 steps: hybridization (which occurs overnight), purification (which takes about 3 hours), and then digital counting (which takes 3-5 hours).[30] The turnaround from the tissue sample arriving at a laboratory to the final result can be as few as 3 days (including processing time for each step). In contrast, other gene expression assays often require several weeks for processing, which involves sending the sample to a central laboratory and waiting for the results to be returned. This substantial reduction in wait time is invaluable for clinicians in the process of making therapeutic decisions with their patients.

To complete the transition of the PAM50 research assay to the clinical nCounter assay, samples from the original PAM50 data set were reanalyzed using the prototypical intrinsic subtype centroids generated from nCounter training. This analysis enables computation of the ROR score and assignment of a risk category. Test samples that are evaluated using the NanoString nCounter system—called the Prosigna Breast Cancer Prognostic Gene Signature Assay—are provided with the Prosigna ROR Score (0-100), the risk category, and the intrinsic subtype.

Studies have been conducted to validate the analytic precision of the assay. Conducting such studies is particularly important for decentralized tests, to ensure reproduc-

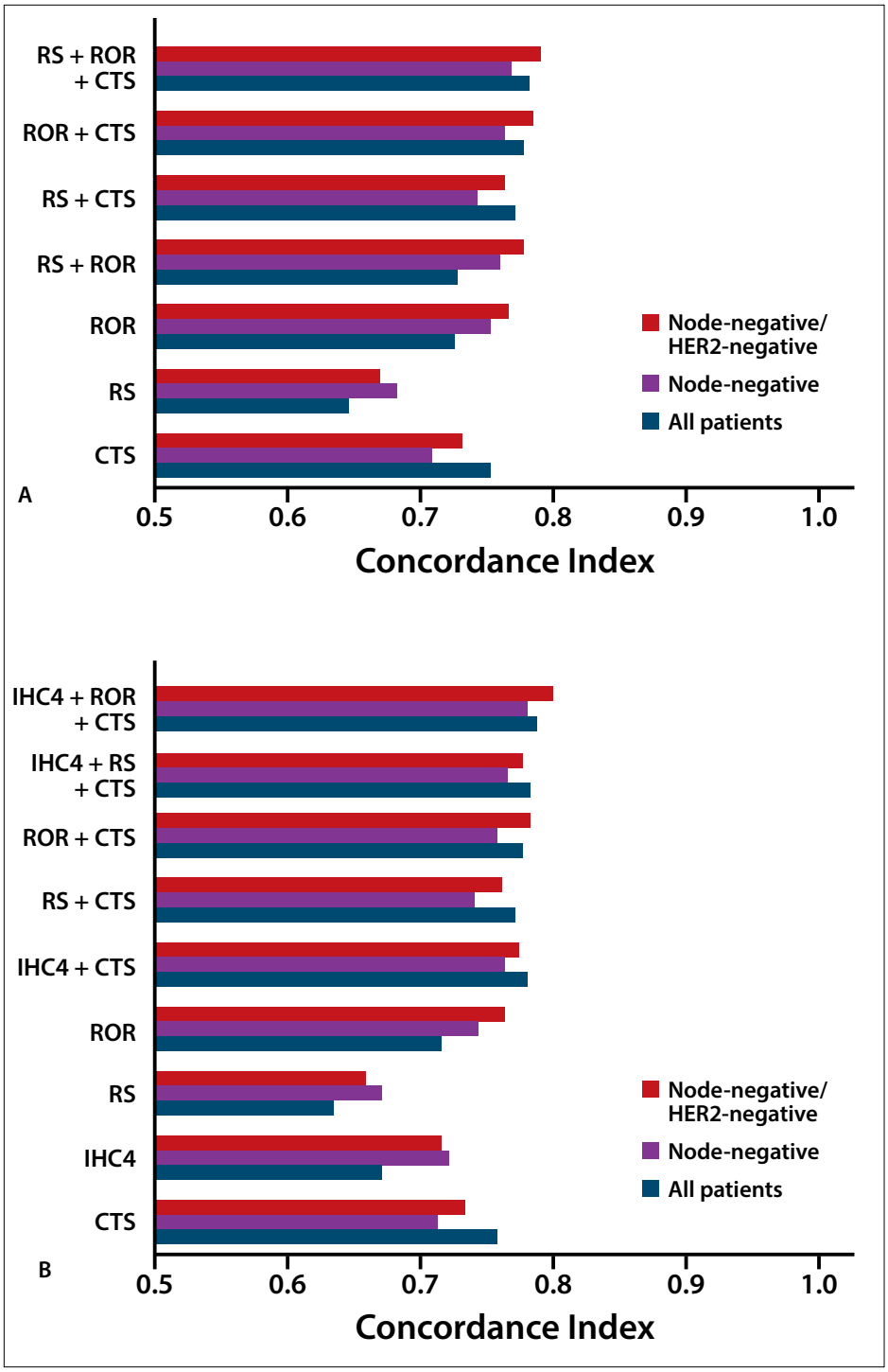


**Figure 3.** Comparison of the concordance index for ROR with (A) RS and (B) IHC4 in a validation study. CTS, clinical treatment score; HER2, human epidermal growth factor receptor 2; IHC, immunohistochemistry; ROR, risk of recurrence; RS, recurrence score. Adapted from Dowsett M et al. *J Clin Oncol.* 2013;31(22):2783-2790.[29]

ibility across individuals and the instrumentation being used. Nielsen and colleagues evaluated the precision and reproducibility of the PAM50 Assay.[31] To evaluate the precision of the assay among testing sites, 5 pooled breast cancer tumor RNA samples representing each intrinsic subtype (2 for Luminal B) were evaluated using the PAM50-based assay at 3 independent centers.[31] Each sample was tested 36 times at each site for a total of 108 replicates for

each sample. For each sample tested, the total standard deviation was less than 1 ROR unit on the 0 to 100 scale. There was complete agreement on the intrinsic subtype and risk group.[31] The reproducibility of the PAM50 Assay was evaluated by performing it on replicate tissue sections from 43 formalin-fixed, paraffin-embedded tumor blocks at 3 sites. In these analyses, the total standard deviation was 2.9 ROR units, indicating that the assay can measure a 6.75-unit difference between 2 ROR scores with 95% confidence.[31] Overall, the precision and reproducibility of the assay as conducted at the 3 test sites were similar to what had been reported for centralized laboratory tests.[31] These findings provide evidence supporting the decentralization of the PAM50 Prosigna Assay.

### *Clinical Validation of the Prosigna Assay*

Several clinical validation studies have been undertaken to evaluate the ability of the Prosigna (PAM50) ROR score to predict the likelihood of distant recurrence in patients with ER-positive primary breast cancer. A validation study by Dowsett and colleagues of patients who received anastrozole or tamoxifen in the ATAC trial compared the prognostic value of the PAM50 Assay against clinical factors, the IHC4, and the Oncotype DX® recurrence score.[29] IHC4 is a composite score that accounts for 4 IHC markers—ER, PR, Ki-67, and HER2—which was developed and optimized on the TransATAC data set. Oncotype DX, a 21-gene assay, is the multigene assay most frequently ordered by oncologists for patients with early-stage breast cancer in the United States. In this validation study of endocrine-treated patients with ER-positive, node-negative breast cancer, the PAM50 ROR score provided significant prognostic information beyond what was provided by conventional clinical factors, the Oncotype DX recurrence score, and a combination of the 2 approaches (Figure 3).[29] Compared with the Oncotype recurrence score, the PAM50 ROR score also categorized more patients in the high-risk category and fewer patients in the intermediate-risk category, thus providing more definitive information. In an analysis by Sestak and colleagues, the PAM50 ROR score distinguished distant recurrence in years 5 to 10 better than the Oncotype recurrence score.[32] Based on the ROR score, 23% of patients in the intermediate-risk group were changed to the high-risk group. Outcome for intermediate patients as identified by the PAM50 ROR score was better, as seen in the Kaplan-Meier curve separation for the intermediate group. Even when tumor size was excluded from the ROR score, the score still provided better prognostic value than the recurrence score from Oncotype. These results suggest that the gene set provided by the PAM50 Assay provides valuable information that can be used in conjunction with tumor size.

A second clinical validation study assessed the prognostic value of the PAM50 ROR score in postmenopausal women with ER-positive breast cancer who received adjuvant endocrine therapy in the ABCSG-8 (Austrian Breast and Colorectal Cancer Study Group) trial.[33] The ABCSG-8 trial evaluated whether a switch to anastrozole after 2 years of tamoxifen would be superior to continued tamoxifen.[34] In the validation study, Gnant and colleagues performed the PAM50 test on tumor samples from 1478 patients enrolled in ABCSG-8 and compared the prognostic value of the ROR score against that obtained with standard clinicopathologic parameters.[33]

The ROR score was found to add significant prognostic value over standard clinical variables. The ROR-based risk groups, which contained a similar number of patients, could clearly discriminate outcomes. The predicted 10-year distant recurrence-free survival rates were 96.7% for the low-risk group, 91.3% for the intermediate-risk group, and 79.9% for the high-risk group (Figure 4).[33] Reflecting previously reported prognostic differences between subtypes, the 10-year distant recurrence-free survival rate was significantly higher for Luminal A vs Luminal B (93.9% vs 82.2%; HR, 2.85; $P<.0001$).[33] Approximately 30% of the patients in this study were node-positive, and the results show a low rate of recurrence even among patients with low-risk, node-positive disease.

These studies provide a robust data set for assessing the likelihood of late recurrence in patients with ER-positive breast cancer throughout a 10-year period. The long length of follow-up is particularly advantageous compared with shorter follow-up periods for patients with luminal-type disease, given the differences in recurrence patterns between Luminal A and Luminal B. For patients with Luminal A disease, the risk of relapse throughout 10 years is approximately 3%.[29] For patients with low-risk PAM50 results, it is valuable to be able to discuss with patients their low risk of relapse, the low likelihood of benefit of chemotherapy, and the toxicities of chemotherapy.

Based on the findings of the validation studies, the Prosigna Assay received US Food and Drug Administration 510(k) clearance in September 2013. The assay received the clearance based on its intended use as a prognostic indicator for distant recurrence-free survival at 10 years for postmenopausal women with stage I/II node-negative or stage II node-positive, hormone receptor–positive breast cancer who have undergone surgery with appropriate locoregional treatment.

In a study presented at the 2015 American Society of Clinical Oncology (ASCO) meeting, Cheang and colleagues used tissue samples from patients in the TNT (Triple Negative Breast Cancer) trial to evaluate the concordance between intrinsic subtypes and ROR groups.[14,35] The basal-like breast cancer and high-risk ROR scores in primary tumors were

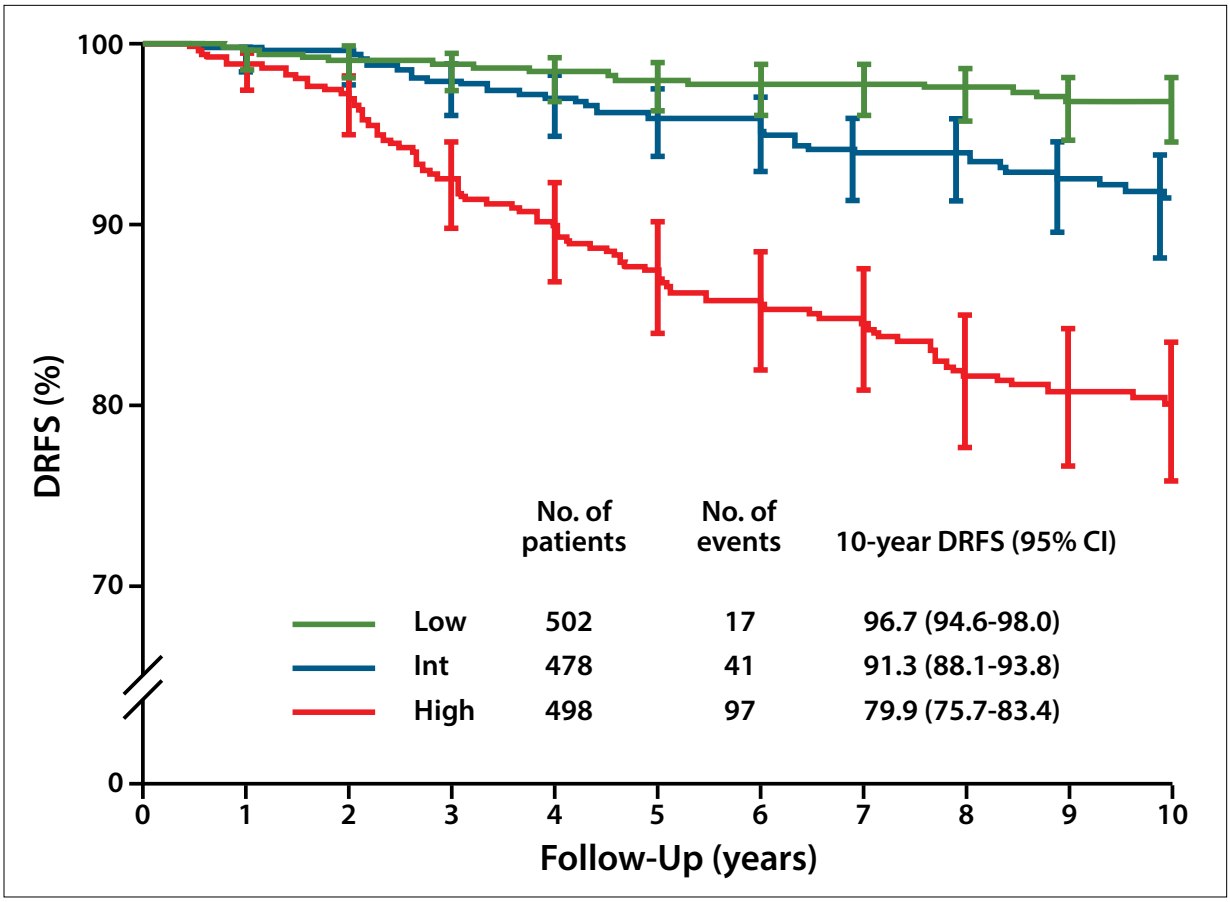


**Figure 4.** Distant recurrence-free survival (DRFS) rates at 10 years according to risk group in an analysis of tumor samples from patients enrolled in the ABCSG-8 trial. ABCSG, Austrian Breast and Colorectal Cancer Study Group. Adapted from Gnant M et al. *Ann Oncol.* 2014;25(2):339-345.[33]

substantially conserved with matched positive lymph nodes and recurrent tissue samples. There were no cases in which a primary tumor/non–basal-like breast cancer was classified as basal-like breast cancer in matched positive lymph nodes or recurrent tissue samples. In matched positive lymph nodes and recurrent tissue samples, the Luminal A subtype appeared to change to an aggressive subtype or a higher ROR-defined risk group.

Three studies presented at the 2015 ASCO meeting evaluated the ability of the PAM50 ROR score to predict disease recurrence.[36-38] These analyses were based on data from the Danish Breast Cancer Cooperative Group. All 3 studies showed that the use of the ROR score improved prediction of distant recurrence. The ROR score helped identify patients, whether node-negative[36] or node-positive,[37] who had an excellent prognosis and could avoid overtreatment with adjuvant chemotherapy and patients who did not require endocrine therapy after 5 years.[38] The recently updated guidelines from the St Gallen International Expert Consensus state that the PAM50 ROR score is clearly prognostic beyond 5 years.[39]

### *Other Potential Applications of the Prosigna (PAM50) Assay*

Studies have evaluated other potential uses of the PAM50 Assay to predict outcomes in breast cancer and help plan adjuvant therapy. One possible application involves the use of PAM50 to predict late distant recurrence after endocrine therapy, which could potentially define which patients might benefit from extended endocrine therapy. In one analysis, Filipits and colleagues performed the PAM50 Assay on tumor samples from 1246 patients enrolled in ABCSG-8 who were alive and disease-free 5 years after diagnosis.[40] The ROR score and ROR-derived risk group stratification both provided significantly more prognostic information for predicting late distant recurrence than a combination of clinical factors ($P$<.001 for both). The risk of distant recurrence in years 5 to 15 ranged from 2.4% in the low-risk group to 17.5% in the high-risk group. These trends were observed in both node-positive and node-negative disease.[40]

Subsequently, a combined analysis was undertaken using a larger set of patients from the ATAC and

ABCSG-8 trials.[41] Prosigna (PAM50) Assay results and clinical outcomes were evaluated in 2137 postmenopausal women with hormone receptor–positive, early-stage breast cancer without recurrence 5 years after diagnosis. The cutoff ranges were based on the 10-year risk of distant recurrence: low, less than 10%; intermediate, 10% to 20%; high, greater than 20%. (It should be noted that different studies employ different cutoff points to designate low, intermediate, and high risk.) In this analysis, the clinical treatment score was the strongest prognostic factor, although the ROR score was significantly prognostic in years 5 to 10. Moreover, the ROR score had more prognostic value than the clinical treatment score within the subset of patients with HER2-negative, node-negative disease.[41] Overall, 25% of patients with node-positive disease were classified in the low-risk ROR group, in which the risk of distant recurrence in years 5 to 10 was 3.3%.[41] This low risk of distant recurrence in the 10-year period brings into question the role of adjuvant chemotherapy and the use of extended endocrine therapy in these patients. Conversely, the high-risk ROR category included some women with node-negative disease.

The Prosigna (PAM50) Assay has also been evaluated for its ability to predict local recurrence in patients with early breast cancer. In 2014, Fitzal and colleagues reported results from an analysis of the ABCSG-8 trial.[42] Tumor samples were analyzed from 1308 patients, and outcomes were evaluated after a median follow-up of 11 years. In a Cox regression model, the ROR score was significantly predictive of local recurrence, with 10-year local recurrence-free survival rates ranging from 98.4% in low-risk patients to 94.4% in high-risk patients ($P$=.0005).[42] The intrinsic subtype was also an independent predictor for local recurrence-free survival. These preliminary studies suggest other potential applications of the Prosigna Assay in tailoring therapy for patients with breast cancer.

New studies examining the utility of the PAM50 Assay to predict responses to chemotherapy were presented at the 2015 ASCO meeting. In an analysis by Rodriguez and coworkers, the Prosigna ROR score significantly predicted response to neoadjuvant chemotherapy.[43] Among the intrinsic subtypes, Luminal A tumors were the least responsive to neoadjuvant chemotherapy, a finding that supports the recently updated St Gallen guidelines.[39] A decision-impact study by Wuerstlein and associates evaluated how the Prosigna PAM50 Assay affected patients' and physicians' perceptions of treatment choice after they received the Prosigna test results.[44] A 29.3% discordance in intrinsic subtyping was found between the PAM50 Assay and IHC. Results from the Prosigna Assay changed the selection of chemotherapy in 36 patients (18.2%), including 36.7% of patients classified as Luminal B by PAM50 testing.[44] This analysis confirms the findings of GEICAM, a similar study in which Prosigna significantly changed treatment recommendations while increasing physician confidence in the test and reducing patient anxiety.[45]

## Conclusion

Data suggest that intrinsic subtype may be able to provide important information regarding the disease course and, potentially, response to treatment.[14,24] For example, Tutt and colleagues found that the efficacy of carboplatin was stronger in patients with *BRCA1/2* mutations, and that patients with nonbasal triple-negative breast cancer responded better to a taxane than a platinum.[14] In a study by Martín and coworkers, only the subset of patients with a low PAM50 proliferation score had a benefit from paclitaxel.[24] Ongoing research is attempting to elucidate how best to apply molecular signatures in the management of patients with breast cancer.

***Disclosure***

*Dr Boccia is a speaker for NanoString Technologies, Inc., which offers the Prosigna® Breast Cancer Prognostic Gene Signature Assay, based on the PAM50 gene signature.*

## References


1. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature*. 2000;406(6797):747-752.
2. Sørlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A*. 2001;98(19):10869-10874.
3. Hu Z, Fan C, Oh DS, et al. The molecular portraits of breast tumors are conserved across microarray platforms. *BMC Genomics*. 2006;7:96.
4. Perreard L, Fan C, Quackenbush JF, et al. Classification and risk stratification of invasive breast carcinomas using a real-time quantitative RT-PCR assay. *Breast Cancer Res*. 2006;8(2):R23.
5. Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. *Mol Oncol*. 2011;5(1):5-23.
6. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490(7418):61-70.
7. Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol*. 2009;27(8):1160-1167.
8. Tibshirani R, Hastie T, Narasimhan B, Chu G. Diagnosis of multiple cancer types by shrunken centroids of gene expression. *Proc Natl Acad Sci U S A*. 2002;99(10):6567-6572.
9. Bastien RR, Rodríguez-Lescure Á, Ebbert MT, et al. PAM50 breast cancer subtyping by RT-qPCR and concordance with standard clinical molecular markers. *BMC Med Genomics*. 2012;5:44.
10. German Association of Gynecological Oncology. Diagnosis and Treatment of Patients with Primary and Metastatic Breast Cancer. Version 2015.1. http://www.ago-online.de/fileadmin/downloads/leitlinien/mamma/maerz2015/en/2015E_Updated_Guidelines.pdf. Posted March 2015. Accessed June 2, 2015.
11. Goldhirsch A, Winer EP, Coates AS, et al; Panel members. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol*. 2013;24(9):2206-2223.
12. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: breast cancer. Version 2.2015. Updated March 11, 2015. http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Accessed April 11, 2015.
13. Prat A, Lluch A, Albanell J, et al. Predicting response and survival in chemotherapy-treated triple-negative breast cancer. *Br J Cancer*. 2014;111(8):1532-1541.

14. Tutt A, Ellis P, Kilburn L, et al. TNT: a randomized phase III trial of carboplatin (C) compared with docetaxel (D) for patients with metastatic or recurrent locally advanced triple negative or *BRCA1/2* breast cancer (CRUK/07/012). Paper presented at: 37th Annual San Antonio Breast Cancer Symposium; December 9-13, 2014; San Antonio, Texas. Abstract S3-01.
15. Glück S, de Snoo F, Peeters J, Stork-Sloots L, Somlo G. Molecular subtyping of early-stage breast cancer identifies a group of patients who do not benefit from neoadjuvant chemotherapy. *Breast Cancer Res Treat*. 2013;139(3):759-767.
16. Nielsen TO, Parker JS, Leung S, et al. A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. *Clin Cancer Res*. 2010;16(21):5222-5232.
17. Chia SK, Bramwell VH, Tu D, et al. A 50-gene intrinsic subtype classifier for prognosis and prediction of benefit from adjuvant tamoxifen. *Clin Cancer Res*. 2012;18(16):4465-4472.
18. Jørgensen CL, Nielsen TO, Bjerre KD, et al. PAM50 breast cancer intrinsic subtypes and effect of gemcitabine in advanced breast cancer patients. *Acta Oncol*. 2014;53(6):776-787.
19. Conforti R, Boulet T, Tomasic G, et al. Breast cancer molecular subclassification and estrogen receptor expression to predict efficacy of adjuvant anthracyclines-based chemotherapy: a biomarker study from two randomized trials. *Ann Oncol*. 2007;18(9):1477-1483.
20. Colleoni M, Cole BF, Viale G, et al. Classical cyclophosphamide, methotrexate, and fluorouracil chemotherapy is more effective in triple-negative, node-negative breast cancer: results from two randomized trials of adjuvant chemoendocrine therapy for node-negative breast cancer. *J Clin Oncol*. 2010;28(18):2966-2973.
21. Pritchard KI, Shepherd LE, O'Malley FP, et al; National Cancer Institute of Canada Clinical Trials Group. HER2 and responsiveness of breast cancer to adjuvant chemotherapy. *N Engl J Med*. 2006;354(20):2103-2111.
22. Cheang MC, Voduc KD, Tu D, et al. Responsiveness of intrinsic subtypes to adjuvant anthracycline substitution in the NCIC.CTG MA.5 randomized trial. *Clin Cancer Res.* 2012;18(8):2402-2412.
23. Levine MN, Pritchard KI, Bramwell VH, Shepherd LE, Tu D, Paul N; National Cancer Institute of Canada Clinical Trials Group. Randomized trial comparing cyclophosphamide, epirubicin, and fluorouracil with cyclophosphamide, methotrexate, and fluorouracil in premenopausal women with node-positive breast cancer: update of National Cancer Institute of Canada Clinical Trials Group Trial MA5. *J Clin Oncol*. 2005;23(22):5166-5170.
24. Martín M, Prat A, Rodríguez-Lescure A, et al. PAM50 proliferation score as a predictor of weekly paclitaxel benefit in breast cancer. *Breast Cancer Res Treat*. 2013;138(2):457-466.
25. Martín M, Rodríguez-Lescure A, Ruiz A, et al; GEICAM 9906 Study Investigators. Randomized phase 3 trial of fluorouracil, epirubicin, and cyclophosphamide alone or followed by paclitaxel for early breast cancer. *J Natl Cancer Inst*. 2008;100(11):805-814.
26. Winer EP, Berry DA, Woolf S, et al. Failure of higher-dose paclitaxel to improve outcome in patients with metastatic breast cancer: Cancer and Leukemia Group B trial 9342. *J Clin Oncol.* 2004;22(11):2061-2018.
27. Seidman AD, Berry D, Cirrincione C, et al. Randomized phase III trial of weekly compared with every-3-weeks paclitaxel for metastatic breast cancer, with trastuzumab for all HER-2 overexpressors and random assignment to trastuzumab or not in HER-2 nonoverexpressors: final results of Cancer and Leukemia Group B protocol 9840. *J Clin Oncol*. 2008;26(10):1642-1649.
28. Prat A, Bianchini G, Thomas M, et al. Research-based PAM50 subtype predictor identifies higher responses and improved survival outcomes in HER2-positive breast cancer in the NOAH study. *Clin Cancer Res*. 2014;20(2):511-521.
29. Dowsett M, Sestak I, Lopez-Knowles E, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. *J Clin Oncol*. 2013;31(22):2783-2790.
30. Prosigna Breast Cancer Prognostic Gene Signature Assay [package insert]. Seattle, WA: NanoString Technologies, Inc; 2015.
31. Nielsen T, Wallden B, Schaper C, et al. Analytical validation of the PAM50-based Prosigna Breast Cancer Prognostic Gene Signature Assay and nCounter Analysis System using formalin-fixed paraffin-embedded breast tumor specimens. *BMC Cancer*. 2014;14:177.
32. Sestak I, Dowsett M, Zabaglo L, et al. Factors predicting late recurrence for estrogen receptor-positive breast cancer. *J Natl Cancer Inst*. 2013;105(19):1504-1511.
33. Gnant M, Filipits M, Greil R, et al; Austrian Breast and Colorectal Cancer Study Group. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 Risk of Recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. *Ann Oncol*. 2014;25(2):339-345.
34. Jonat W, Gnant M, Boccardo F, et al. Effectiveness of switching from adjuvant tamoxifen to anastrozole in postmenopausal women with hormone-sensitive early-stage breast cancer: a meta-analysis. *Lancet Oncol*. 2006;7(12):991-996.
35. Cheang MCU, Bliss J, Dowsett M, et al. Concordance of intrinsic subtyping and risk of recurrence (ROR) scores between matched primary and metastatic tissue from Triple Negative Breast Cancer Trial (TNT) [ASCO abstract 1019]. *J Clin Oncol*. 2015;33(suppl).
36. Lænkholm A-V, Jensen M-B, Eriksen JO, et al. Prediction of 10yr distant recurrence (DR) using the Prosigna (PAM50) assay in a Danish Breast Cancer Cooperative Group (DBCG) cohort of postmenopausal Danish women with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5yr of endocrine therapy (ET) alone. [ASCO abstract 546]. *J Clin Oncol*. 2015;33(suppl).
37. Ejlertsen B, Jensen M-B, Eriksen JO, et al. Validation of prediction of distant recurrence (DR) by Prosigna (PAM50) in subgroups of a Danish Breast Cancer Cooperative Group (DBCG) cohort of node-positive (N1), hormone receptor positive (HR+), postmenopausal early breast cancer (EBC) patients allocated 5yr of endocrine therapy (ET) [ASCO abstract 513]. *J Clin Oncol*. 2015;33(suppl).
38. Lænkholm A-V, Jensen M-B, Eriksen JO, et al. Prediction of late distant recurrence (DR) using the Prosigna (PAM50) test in a Danish Breast Cancer Cooperative Group (DBCG) cohort of postmenopausal women diagnosed with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5yr of endocrine therapy (ET) [ASCO abstract 544]. *J Clin Oncol*. 2015;33(suppl).
39. Coates AS, Winer EP, Goldhirsch A, et al; Panel members. Tailoring therapies—improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015 [published online May 4, 2015]. *Ann Oncol*. 2015. mdv221.
40. Filipits M, Nielsen TO, Rudas M, et al; Austrian Breast and Colorectal Cancer Study Group. The PAM50 risk-of-recurrence score predicts risk for late distant recurrence after endocrine therapy in postmenopausal women with endocrine-responsive early breast cancer. *Clin Cancer Res*. 2014;20(5):1298-1305.
41. Sestak I, Cuzick J, Dowsett M, et al. Prediction of late distant recurrence after 5 years of endocrine treatment: a combined analysis of patients from the Austrian breast and colorectal cancer study group 8 and arimidex, tamoxifen alone or in combination randomized trials using the PAM50 risk of recurrence score. *J Clin Oncol*. 2015;33(8):916-922.
42. Fitzal F, Filipits M, Fesl C, et al. Predicting local recurrence using PAM50 in postmenopausal endocrine responsive breast cancer patients [ASCO abstract 1008]. *J Clin Oncol*. 2014;32:5s(suppl).
43. Rodriguez BJ, Fernandez AIL, Ribelles N, et al. Prosigna (PAM50) to predict response to neoadjuvant chemotherapy (NAC) in HR+/HER2- early breast cancer (EBC) patients [ASCO abstract 11049]. *J Clin Oncol*. 2015;33(suppl).
44. Wuerstlein R, Sotlar K, Gluz O, et al. Significance of prospective multicenter decision impact WSG-BCIST Study in postmenopausal ER+ HER2- N0 early breast cancer (EBC) for molecular testing for intrinsic subtype definition [ASCO abstract 535]. *J Clin Oncol*. 2015;33(suppl).
45. Martín M, Gonzalez-Rivera M, Morales S, et al. Prospective study of the impact of the Prosigna assay on adjuvant clinical decision-making in unselected patients with estrogen receptor positive, human epidermal growth factor receptor negative, node negative early-stage breast cancer. *Curr Med Res Opin*. 2015;31(6):1129-1137.

# A Comparison of Breast Cancer Multianalyte Assays With Algorithmic Analyses (MAAA) for Their Net Predictive/Prognostic Value

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**Abstract:** In breast cancer, prognostic and predictive information has traditionally been ascertained using clinicopathologic measures, such as tumor size and grade, lymph node involvement, and the presence of protein biomarkers, including the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2)/neu. These parameters provide important prognostic and predictive information. Conventional clinicopathologic factors also help guide the use of adjuvant therapy. However, standard clinicopathologic factors have a limited ability to estimate prognosis, predict responses to chemotherapy, and help guide the selection of chemotherapeutic agents. In addition, clinical factors alone can be misleading in that they do not fully indicate whether chemotherapy is needed or if hormonal treatment in ER-positive patients is sufficient. Multianalyte assays with algorithmic analyses (MAAA) were developed toward the goal of personalized medicine, to supplement existing techniques and further inform the decision of whether to treat with adjuvant chemotherapy. Several gene expression profiling tests are now available for use in patients with breast cancer. In general, these tests provide an estimate of prognosis. In select instances, they may also be predictive for benefit from chemotherapy. This article reviews the latest studies evaluating the use of these tests.

Multianalyte assays with algorithmic analyses (MAAA) were developed toward the goal of personalized medicine, in which a patient's therapy is tailored based on the unique characteristics of his or her condition. In cancer therapy, MAAAs are used to analyze a tumor to gain prognostic information (estimating the patient's likely clinical outcome) and, potentially, to gain predictive information (estimating the likely benefit from specific therapies).

In breast cancer, prognostic and predictive information has traditionally been ascertained using clinicopathologic measures, such as tumor size and grade, lymph node involvement, and the presence of protein biomarkers including the estrogen receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2)/neu. These parameters provide important prognostic and predictive information. For example, a high tumor grade as assessed by the Nottingham Grading system is strongly prognostic, as it is associated with shorter breast cancer–specific survival and disease-free survival.[1] Distant recurrence in patients with high-grade tumors tends to occur within 8 years of diagnosis. The significance of tumor grade as a prognostic factor reflects the importance of cell proliferation. High-grade tumors demonstrate high proliferation rates, which are strongly associated with poor prognosis regardless of how it is measured.[2] The rapid proliferation rate of high-grade tumors also makes these tumors more susceptible to chemotherapy, which kills cells in the proliferation phase of the cell cycle. Given the availability of multiple chemotherapeutic agents that attack different components of the proliferation cycle, chemotherapy can be particularly effective in high-grade tumors, delaying tumor recurrence and improving outcomes.

Conventional clinicopathologic factors also help guide the use of adjuvant therapy.[3-5] Hormone receptor–positive tumors are likely to be responsive to endocrine therapy, and patients with HER2-positive tumors receive HER2/neu-targeted therapy.[3-5] Algorithms have also been developed to consider other clinicopathologic factors such as age, tumor size, and nodal involvement to help estimate the probability of recurrence and guide the use of

adjuvant chemotherapy. Adjuvant! Online (http://www.adjuvantonline.com) is an internet-based risk assessment calculator that estimates a patient's 10-year survival probability, risk of relapse, and expected benefit of adjuvant therapy based on multiple factors, including patient age, menopausal status, comorbidities, tumor stage, tumor size, number of positive nodes, and ER status.[6] The Adjuvant! Online algorithm has been well validated and is thus a valuable tool.[7] However, standard clinicopathologic factors have a limited ability to estimate prognosis, predict responses to chemotherapy, and help guide the selection of chemotherapeutic agents. In addition, clinical factors alone can be misleading in that they do not fully indicate whether chemotherapy is needed or if hormonal treatment in ER-positive patients is sufficient. Gene expression studies were undertaken in an attempt to supplement these techniques and further inform the decision of whether to treat with adjuvant chemotherapy.

Several gene expression profiling tests are now available for use in patients with breast cancer. In general, these tests yield numerical scores that indicate the patient's risk of distant recurrence, providing an estimate of prognosis. To some degree, they recapitulate the work of tumor grade for estimating clinical outcomes, as they contain multiple genes related to cell proliferation. However, because gene expression profiling tests also measure other relevant genes related to cancer growth and survival, they aim to provide additional information beyond tumor grade alone.

The assays have also been developed with the hope of providing predictive value for estimating the benefit of chemotherapy. However, an important caveat with these assays is that because the analyses have been performed on populations of patients and with many different types of chemotherapy regimens, the data are population-based rather than individualized. Some information is known about the populations—for example, some assays have been based on hormone receptor–positive patients, whereas others include both hormone receptor–positive and hormone receptor–negative patients. Moreover, some studies have specified menopausal status, whereas others have not. In this way, results from gene expression assays are not for use in personalized medicine, but they are somewhat similar to the results attained with the Nottingham Grading System.

Despite these scientific limitations, some gene expression profiling tests have been marketed to suggest that they may improve upon the subjectivity inherent in pathologists' grading of tumors. However, each of the gene expression tests should be evaluated individually, as they apply to different populations, evaluate different genes, and are based on different platforms. As a result, there is substantial variation among the available tests. In fact, different assays may yield different outcomes for the same patient: one may yield a low-risk result, whereas another can yield intermediate or even high risk. This discordance can be confusing for clinicians.

All of these tests are prognostic.[8-13] It is important to note that some of these tests may be predictive for benefit from chemotherapy in select instances. The PAM50 Risk of Recurrence (ROR) score has been shown to be predictive for chemotherapy benefit in select instances based on the PAM50 ROR score and intrinsic subtype.[14-20] The Oncotype DX® test and MammaPrint® test both show a strong degree of association between high test scores and chemotherapy benefit,[10,21] which is not surprising, since the vast majority of tumors with high scores are high-grade tumors by histopathology.[22] Most high-grade tumors are expected to respond by virtue of their high proliferation indices.[22]

## Available Gene Signature Assays

### *Prosigna®*

Several gene expression assays are available. The Prosigna Breast Cancer Prognostic Gene Signature Assay test is based on the characterization of 50 genes relevant to breast cancer biology. The assay is based on a 50-gene signature identified by Perou and colleagues that identified 4 main breast cancer intrinsic subtypes.[23] Luminal A tumors are hormone receptor–positive tumors characterized by low proliferation, high hormone receptor expression, and HER2 negativity.[16] These tumors, which are associated with a very good prognosis and a low ROR, are typically Nottingham grade 1. Luminal B tumors are also hormone receptor–positive but are associated with high proliferation rates and tend to be Nottingham grade 2 to 3.[16] These patients would be eligible for endocrine therapy and may also be candidates for chemotherapy.

Basal-like tumors are hormone receptor–positive and show activation of pathways involved in driving proliferation and early breast cancer recurrence.[16] The fourth category, HER2-enriched tumors, are not defined based on HER2 positivity at the gene expression profiling level but rather show activation in pathways downstream from HER2.[16] HER2-enriched tumors tend to show high proliferation and a poor prognosis, with shorter times to local and distant recurrence (5-8 years).

These 4 intrinsic subtypes form the basis of the Prosigna test. The 50-gene set includes a very robust signature for proliferation, with 19 genes targeting the cell cycle.[24] The Prosigna ROR score is based on an algorithm that encompasses not only the PAM50 genes, but also clinicopathologic factors (tumor size and lymph node status) and an average weighting score of the proliferation genes. The Prosigna test was initially developed on a quantitative

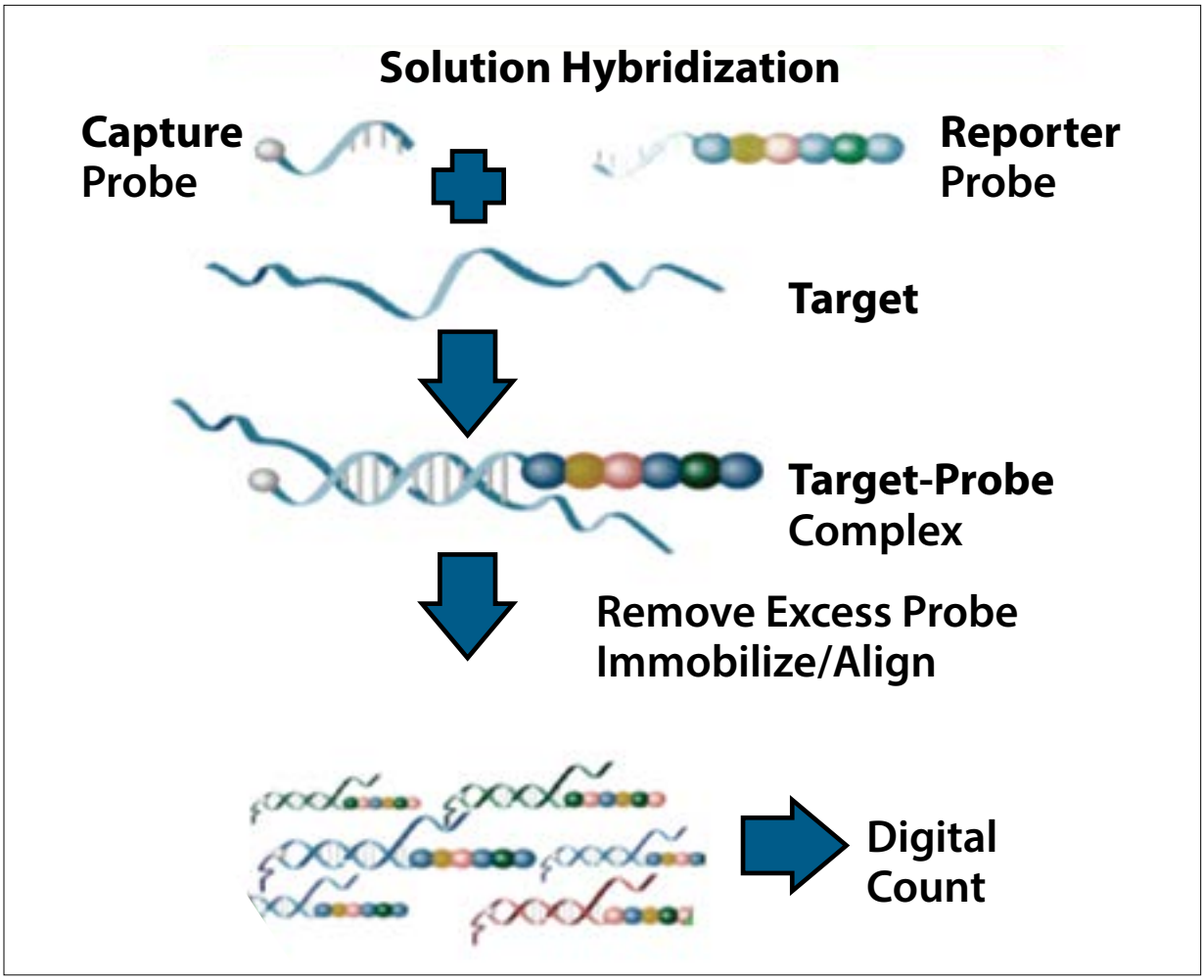


**Figure 1.** Design of the NanoString Technologies nCounter system.

reverse transcriptase polymerase chain reaction (qRT-PCR) platform, but was transferred onto the NanoString Technologies nCounter® system to enable decentralized use of the assay in laboratories around the world. The nCounter system is a very robust platform for detecting gene expression that does not require reverse transcription or nucleic acid amplification (Figure 1). The system uses tagged fluorescent probes that hybridize directly to specific mRNA sequences. These fluorescent probes are then imaged with a fluorescent scanner that digitally counts the abundance of fluorescent probes to determine the mRNA expression level.[24] The assay was initially developed using unsupervised hierarchical clustering on a training set of patients to optimize the method for identifying the intrinsic subtypes. The test was then further refined by combining the biologic information of the intrinsic subtypes into a personalized score—the PAM50 ROR score—which was correlated with patient outcome (Table 1).[18] The PAM50 Assay was transitioned to the nCounter system and further refined to calculate the Prosigna score, based upon a 46-gene subset of the PAM50 genes (removing *BIRC5*, *CCNB1*, *GRB7*, and *MYBL2*). The PAM50 Assay has been analytically validated on the nCounter system, and the assay has shown high concordance with RT-PCR.[25,26] The assay has been validated in several studies. Dowsett and colleagues evaluated tumor samples from 1017 patients who received anastrozole or tamoxifen in the ATAC (Arimidex, Tamoxifen Alone or Combined) trial.[9] The correlation between the 46-gene and 50-gene signature was high (0.998), and results between the 2 gene sets were almost identical irrespective of tumor size inclusion. In another validation study, Gnant and coworkers performed the PAM50 test on tumor samples from 1620 patients enrolled in ABCSG-8 (Austrian Breast and Colorectal Cancer Study Group–8) and compared the prognostic value of the ROR score against that obtained with standard clinicopathologic parameters.[8] The ROR score added significant prognostic value over standard clinical variables, and the ROR-based risk groups had significantly different outcomes. The 10-year distant

**Table 1.** Models of Relapse-Free Survival in Untreated Patients

| | Model A | | Model B | | Model C | |
|---|---|---|---|---|---|---|
| Variable | Hazard Ratio | *P* Value | Hazard Ratio | *P* Value | Hazard Ratio | *P* Value |
| Basal-like[a] | 1.33 | .330 | 1.79 | .030 | 1.58 | .066 |
| HER2-Enriched | 2.53 | .00012 | 3.25 | <.0001 | 2.90 | <.0001 |
| Luminal B[a] | 2.43 | <.0001 | 2.88 | <.0001 | 2.54 | <.0001 |
| ER status[b] | 0.83 | .38 | 0.83 | .34 | 0.83 | .32 |
| Tumor size[c] | 1.36 | .034 | 1.43 | .012 | 1.57 | .001 |
| Node status[d] | 1.75 | .035 | 1.72 | .041 | – | – |
| Histologic grade[e] | 1.40 | .0042 | – | – | – | – |
| Full vs subtype[f] | | <.0001 | | <.0001 | | <.0001 |
| Full vs clinical[g] | | <.0001 | | <.0001 | | <.0001 |

a Luminal A class used as reference state in multivariate analysis.

b Hazard ratios for ER using positive marker in the numerator.

c Size ≤2 cm vs >2 cm.

d Any positive node.

e Grade encoded as an ordinal variable with 3 levels.

f Significant *P* values indicate improved prediction relative to subtype alone.

g Significant *P* values indicate improved prediction relative to clinical data alone.

HER2, human epidermal growth factor receptor 2; ER, estrogen receptor.

Data from Parker JS et al. *J Clin Oncol*. 2009;27(8):1160-1167.[18]

**Table 2.** Prediction of Distant Recurrence by the PAM50 Risk Score, Oncotype DX, and IHC4

| ROR (including tumor size) | Number of Patients | Number of DRs | Comparison | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | CTS (1 *df*) | | ROR (1 *df*) | | RS (1 *df*) | | ROR + CTS vs CTS (1 *df*) | | RS + CTS vs CTS (1 *df*) | |
| | | | LR–$\Delta\chi^2$ | *P* Value | LR–$\chi^2$ | *P* Value | LR–$\chi^2$ | *P* Value | LR–$\Delta\chi^2$ | *P* Value | LR–$\Delta\chi^2$ | *P* Value |
| All patients | 1007 | 160 | 144.9 | <.001 | 99.9 | <.001 | 38.2 | <.001 | 33.9 | <.001 | 22.7 | <.001 |
| Node-negative patients | 739 | 79 | 45.1 | <.001 | 60.9 | <.001 | 28.2 | <.001 | 24.6 | <.001 | 15.0 | <.001 |
| HER2-negative/ node-negative patients | 649 | 62 | 36.6 | <.001 | 53.7 | <.001 | 22.9 | <.001 | 23.4 | <.001 | 10.2 | .001 |

CTS, clinical treatment score; DR, distant recurrence; HER2, human epidermal growth factor receptor 2; LR, likelihood ratio; ROR, risk of recurrence; RS, recurrence score. Data from Dowsett M et al. *J Clin Oncol*. 2013;31(22):2783-2790.[9]

recurrence-free survival rates were predicted to be 96.7% for the low-risk group, 91.3% for the intermediate-risk group, and 79.9% for the high-risk group (Figure 2).[8] The survival rates were also significantly higher for patients with tumors that were Luminal A vs Luminal B (93.9% vs 82.2%; hazard ratio, 2.85; *P*<.0001).[8] Approximately 30% of the patients in this study were node-positive, and the results showed a low rate of recurrence even among patients with low-risk, node-positive disease. The assay met the US Food and Drug Administration (FDA) criteria to allow use of a decentralized model.

These 2 clinical validation studies evaluated the prognostic value of the Prosigna Assay based on 2 different clinical trial registered groups. Together, the 2 analyses included 2495 patients, all postmenopausal women with hormone receptor–positive, node-negative or node-positive disease.[8,9] Taken together, these studies provide Level 1 evidence for the clinical validity of Prosigna.[27] The analyses showed the importance of intrinsic subtypes in predicting outcomes; prognosis was better in the Luminal A subtype than in the other 3 subtypes. A low rate of distant recurrence was seen in low-risk patients, whether

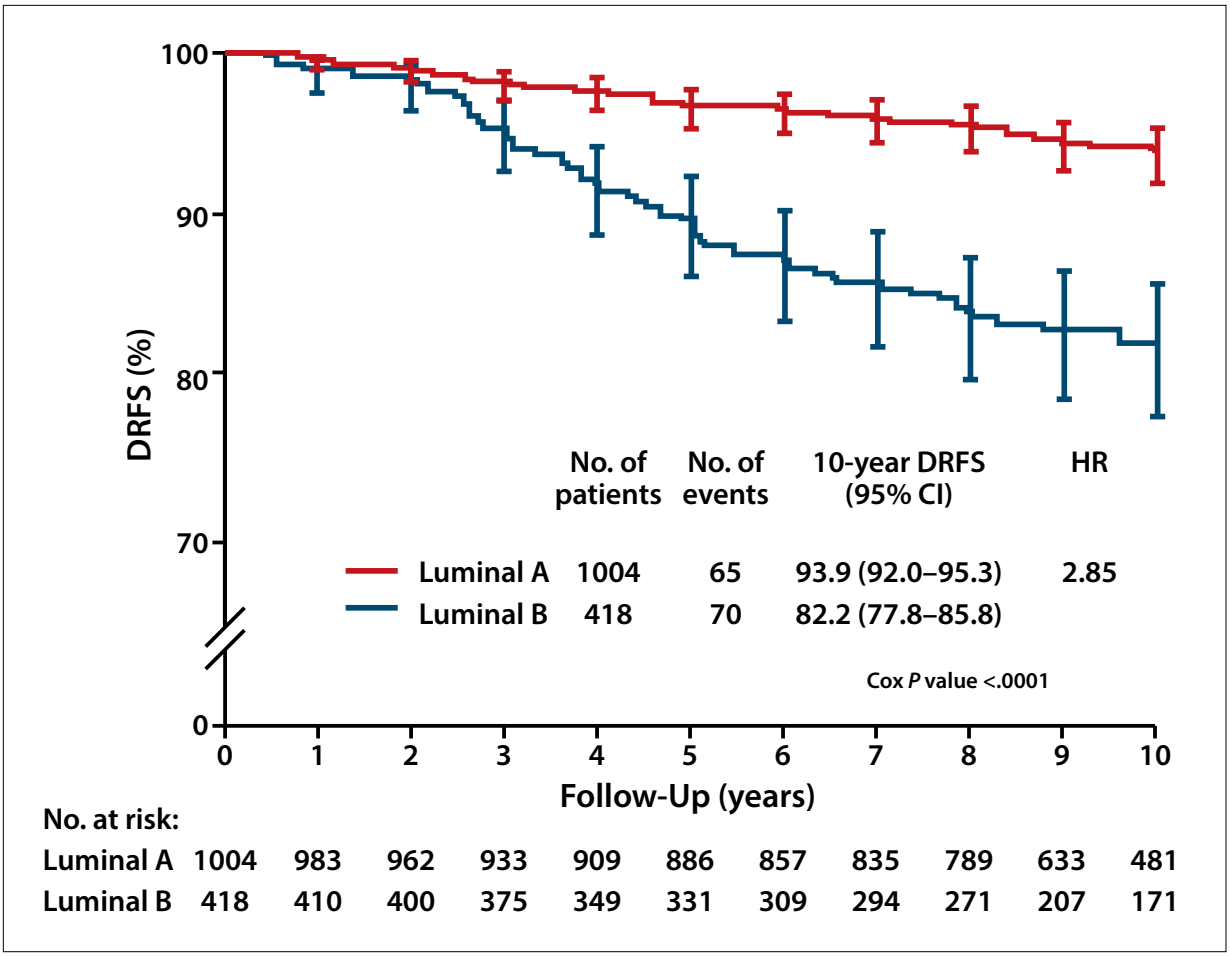


**Figure 2.** Distant recurrence-free survival (DRFS) at 10 years according to luminal subtype. HR, hazard ratio. Adapted from Gnant M et al. *Ann Oncol.* 2014;25(2):339-345.[8]

luminal-positive or luminal-negative. The intrinsic subtypes impart the test with the ability to predict outcomes. Moreover, a head-to-head comparison of different prognostic assays found that the PAM50 ROR provided prognostic information for late distant recurrence significantly beyond what could be obtained with conventional clinicopathologic factors or with another gene signature assay (the Oncotype DX Recurrence Score; Table 2).[9] This analysis used RNA isolated by Genomic Health®, Inc. for a prior study, ATAC, allowing a true head-to-head comparison of the genomic information of the 2 tests. With Prosigna, 23% of patients categorized as intermediate risk by Oncotype were recategorized as high risk. Patients identified as intermediate risk by Prosigna had a better outcome than patients identified as intermediate risk by Oncotype DX.

In a study presented at the 2015 American Society of Clinical Oncology (ASCO) meeting, Cheang and colleagues evaluated the concordance between intrinsic subtypes and ROR groups using tissue samples from patients in the TNT (Triple Negative Breast Cancer) trial.[17,28] The study found that basal-like breast cancer and high-risk ROR scores in primary tumors were substantially conserved with matched positive lymph nodes and recurrent tissue samples. None of the primary tumor/non–basal-like breast cancer cases were classified as basal-like breast cancer in matched positive lymph nodes or recurrent tissue samples. In matched positive lymph nodes and recurrent tissue samples, the Luminal A subtype appeared to change to an aggressive subtype or a higher ROR-defined risk group.

The ability of the PAM50 ROR score to predict disease recurrence was evaluated in several studies presented at the 2015 ASCO meeting.[29-31] In 3 analyses from the Danish Breast Cancer Cooperative Group, use of the ROR score improved prediction of distant recurrence. The studies suggested that the ROR score can help identify patients, both node-negative[29] and node-positive,[30] who have an excellent prognosis and can avoid overtreatment with adjuvant chemotherapy and patients who will not require endocrine therapy beyond 5 years.[31]

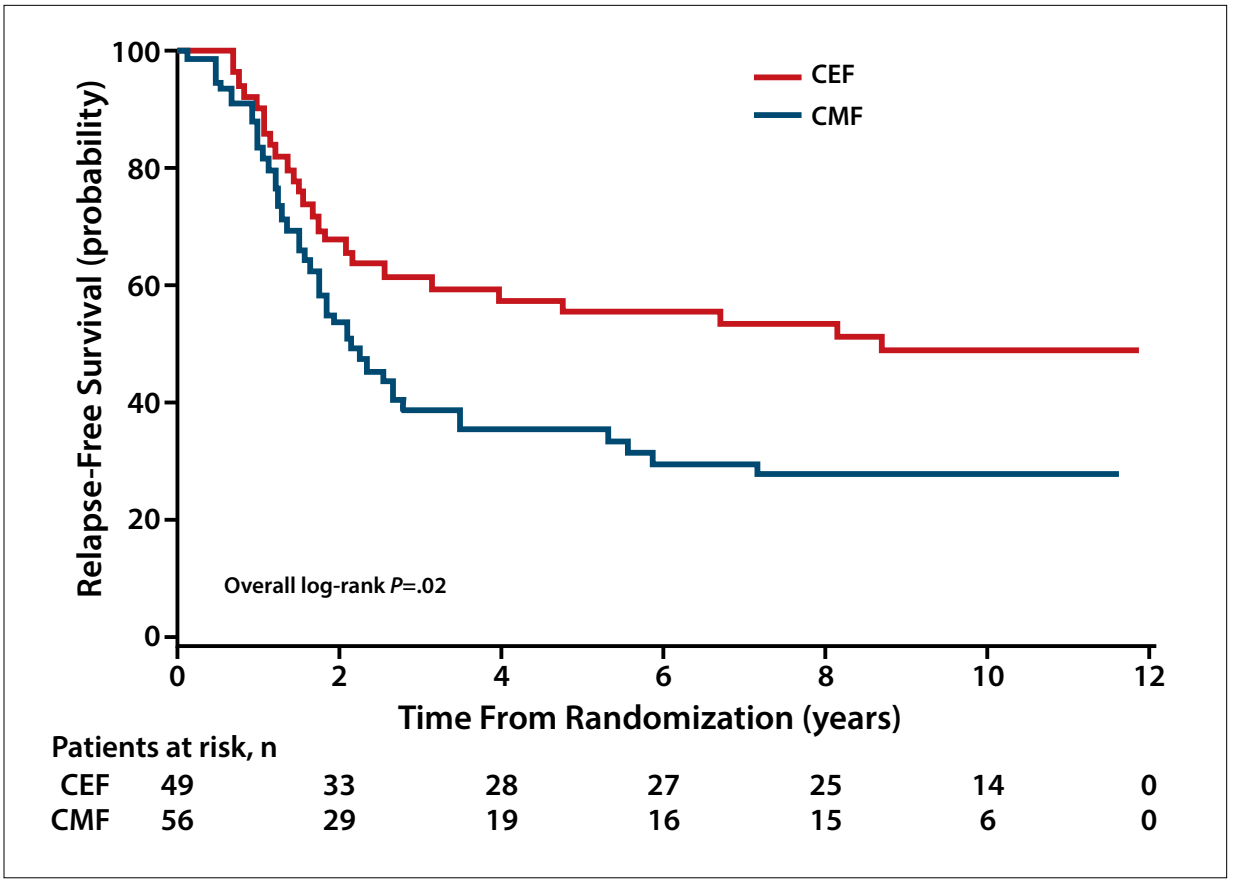


**Figure 3.** Benefit from chemotherapy among HER2-enriched patients in an analysis of tumor samples from the National Cancer Institute of Canada Clinical Trials Group MA.5 trial. CEF, cyclophosphamide/epirubicin/fluorouracil; CMF, cyclophosphamide/methotrexate/fluorouracil. Adapted from Cheang MC et al. *Clin Cancer Res*. 2012;18(8):2402-2414.[32]

Several research studies have demonstrated the potential of the PAM50 intrinsic subtypes to predict response to specific adjuvant therapies.[15,17-19,32,33] In a study by Chia and colleagues, luminal subtypes predicted whether a patient would benefit from tamoxifen in the National Cancer Institute of Canada Clinical Trials Group MA.12 study, but ER status did not.[15] In an analysis by Cheang and coworkers, the HER2-enriched subtype was strongly associated with a greater benefit from anthracycline-based chemotherapy (Figure 3).[32] Prat and associates found that trastuzumab-based chemotherapy was especially beneficial in women with advanced HER2-positive/HER2-enriched tumors and HER2-positive tumors that were predicted to have a high risk of relapse and proliferation status as identified by the PAM50 Assay.[33] A stronger trastuzumab benefit was observed in the HER2-enriched tumors. In a study by Tutt and colleagues, patients with *BRCA1/2* mutations had a stronger response and a longer progression-free survival with carboplatin than patients without the mutation.[17] This study also found that among patients with triple-negative breast cancer, those with the non-basal subtype—as identified by the Prosigna Assay—were more likely to respond to docetaxel than carboplatin.

Studies have also shown the ability of PAM50 to predict response to neoadjuvant chemotherapy.[18,19] In addition to the study by Parker,[18] an evaluation by Rodriguez and colleagues showed that the Prosigna ROR score significantly predicted response to neoadjuvant chemotherapy.[19] Among the intrinsic subtypes, Luminal A tumors were the least responsive to neoadjuvant chemotherapy, a finding that supports the current guidelines from the St Gallen International Expert Consensus, which were updated in May 2015.[34]

Also at the 2015 ASCO meeting, Wuerstlein and associates presented results from a decision impact study that show the impact of the Prosigna PAM50 Assay on patients' and physicians' perceptions on treatment choice after receiving Prosigna test results.[20] There was a 29.3% discordance in intrinsic subtyping between the PAM50 Assay and immunohistochemistry (IHC). Results from the Prosigna Assay changed the chemotherapy decision in 36 patients (18.2%) overall, including 36.7% of PAM50

Luminal B patients.[20] This study confirms the observations of a similarly designed Grupo Español de Investigación en Cáncer de Mama (GEICAM) study, which showed that Prosigna significantly changed treatment recommendations while both increasing physicians' confidence in the test and reducing patient anxiety.[35]

The Prosigna Assay received FDA clearance in September 2013, with an indication specifically for use in postmenopausal women with hormone receptor–positive breast cancer. In December 2014, the assay received an updated FDA clearance for its ability to assess a patient's probability of recurrence between years 5 to 10 after diagnosis, which may help identify patients who may benefit from extended endocrine therapy beyond the first 5 years.[24] This new indication was approved based on an analysis from the ABCSG-8 trial showing that a low PAM50 ROR score was associated with a low absolute risk of distant recurrence (2.4%) in years 5 to 15 of endocrine therapy.[36] These data on Prosigna replicated observations from the TransATAC study,[37,38] generating level 1 evidence based on the way the studies were performed, the duration of follow-up, and the outcomes.[8]

The updated St Gallen guidelines state that the PAM50 ROR score is prognostic beyond 5 years.[34] In March 2015, guidelines from the German Association of Gynecological Oncology added use of the Prosigna Assay for testing newly diagnosed patients with node-negative or node-positive, HR-positive, HER2-negative, early-stage breast cancer with clinicopathologic factors that do not indicate a clear therapeutic decision.[39]

### *Oncotype DX*

The Oncotype DX test is a multigene assay that uses a signature that was developed from genomic databases available in 2000. The assay is based on a qRT-PCR platform and quantifies expression of 16 genes, most of which are related to the hormone receptor as well as proliferation and *HER2*; 5 genes are included for normalization.[40] The test, launched in 2004, was based on an initial training set of patients from the control arm of the National Surgical Adjuvant Breast and Bowel Project B-20 (NSABP) study.[40] Expression of the 21 genes is converted into a recurrence score (0-100), which is reported as a continuous variable and also categorized as low risk (<18), intermediate risk (18-30), and high risk (>30).[10]

In a validation study of patients from the chemotherapy arm of NSABP B-20, the recurrence score was significantly prognostic and appeared to be predictive, with a high-risk recurrence score being significantly associated with a benefit from chemotherapy.[10] However, there are some substantial limitations of this analysis. First, although the patient population was similar in that all patients had hormone receptor–positive disease, there was some heterogeneity in the group based on menopausal status. Moreover, because *HER2* had not yet been identified at the time of trial registration, both trials included a population of unidentified *HER2*-positive patients that has been estimated to be at least 10%.

Another important limitation of the recurrence score data involves a mixing of training and validation sets between the populations of patients enrolled in NSABP B-20, as samples from B-20 were used to select the genes, design the algorithm, and validate the assay.[10,40] This design creates statistical issues in the analysis. In an analysis of the B-20 validation study, Ioannidis illustrated that when validation is properly performed—by not mixing the training and validation sets—the Oncotype DX test is not predictive for chemotherapy benefit.[41] Additionally, to revisit the importance of proliferation, independent studies have shown that the vast majority of high-risk recurrence score tumors are also high grade as assessed by the Nottingham grading system.[42] It stands to reason, therefore, that tumors with a high-risk recurrence score will probably benefit most from the chemotherapy because they typically have very high proliferation rates.

There have also been issues regarding discordance between *HER2* expression as identified by the Oncotype DX single-gene test vs conventional methods (IHC and fluorescence in situ hybridization [FISH]). In 2011, my colleagues and I published an independent analysis showing a false-negative rate of 72% with the HER2 RT-PCR results obtained from Genomic Health Inc., compared with those obtained using IHC and FISH at 3 independent laboratories.[43] This issue was also discovered by other laboratories.[44,45] Independent studies have shown high concordance between IHC and Oncotype DX for ER and PR expression.[45] The issue regarding HER2 concordance has never been properly addressed by the company. Nevertheless, the ASCO/College of American Pathologists committee has recommended against any RT-PCR testing for HER2 for therapeutic purposes.

Oncotype is, however, recommended in the National Comprehensive Cancer Network Breast Cancer guidelines with category 2A evidence.[46] Albain and colleagues evaluated data from Southwest Oncology Group 8814, a phase 3, open-label trial that randomly assigned postmenopausal women with hormone receptor–positive, node-positive breast cancer to receive cyclophosphamide, doxorubicin, and fluorouracil with concurrent or sequential tamoxifen or tamoxifen alone.[47] The analysis found that chemotherapy was likely to benefit patients with a high-risk recurrence score.[47]

The TAILORx (Trial Assigning Individualized Options for Treatment [Rx]) trial is evaluating the effect of chemotherapy in patients with a midrange recurrence score.[48] It is enrolling 11,248 patients. The recurrence score criteria in the

TAILORx trial will differ from that used in the validation trials. A low recurrence score will be 10 or less, intermediate will be 11 to 25, and high will be 26 or greater.

***MammaPrint***

The MammaPrint 70-gene breast cancer recurrence assay is a microarray-based multigene assay that measures expression of mRNA levels in tumor samples based on a gene set first reported by van't Veer and colleagues in 2002.[11] The gene expression profile was identified starting from a whole genome analysis of approximately 25,000 genes using 78 lymph node–negative breast cancer tumor samples from untreated patients. The final set of 70 genes was identified based on its significant association with the risk of breast cancer recurrence, which was developed in 34 patients.[11] This study served as initial validation of the 70-gene assay. A unique feature of the MammaPrint test is that it does not report a numerical recurrence score but rather categorizes patients only as having a good-prognosis signature or a poor-prognosis signature (based on how closely a patient's signature correlates to the good-prognosis signature).[12] This test defines risk based on the chance of distant recurrence at 10 years; low risk is approximately 10% and high risk is 29%.

In another validation study, van de Vivjer and colleagues evaluated the prognostic value of the MammaPrint assay in a series of 295 consecutive patients with stage I or II disease (node-negative or node-positive).[12] The MammaPrint assay was a strong independent predictor of disease outcome, yielding 10-year distant recurrence-free survival rates of 50.6% in the poor-prognosis group and 85.2% in the good-prognosis group, for an estimated hazard ratio of progression of 5.1 (95% CI, 2.9-9.0; $P<.001$).[12]

Based on these studies, the FDA cleared MammaPrint for use in women with node-negative stage 1 or 2 invasive breast cancer with a tumor size of 5.0 cm or less. However, the assay provides little information regarding specific prognosis for individual patients.

Until recently, MammaPrint was approved for use only in fresh or frozen tissue. This requirement, along with the fact that the only laboratory running the assays was located in the Netherlands, prevented the widespread adoption of the assay in the United States. However, in February 2015, the FDA granted Agendia 510(k) clearance to perform the MammaPrint test in formalin-fixed, paraffin-embedded tissue. With the ability to use preserved tissue and the addition of a laboratory in Irvine, California, the assay may gain more traction in the United States.

Agendia is currently undertaking an important large trial to further evaluate the clinical utility of the MammaPrint test. The randomized, prospective phase 3 MINDACT (Microarray in Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy) trial aims to determine the value added by MammaPrint over current clinicopathologic parameters and compared with the Adjuvant! Online Tool.[49] The trial accrued 6600 patients between 2006 and 2011. Patients with a low-risk MammaPrint profile who have a good prognostic index, or are considered low-risk by Adjuvant! Online, will be considered to be concordant in their testing and will receive endocrine therapy only. Patients who have a high-risk MammaPrint test result and high-risk parameters on Adjuvant! Online will receive chemotherapy (and endocrine therapy if they are also hormone receptor–positive). In cases in which there is discordance between the tests—for example, a low-risk MammaPrint and a high-risk Adjuvant! Online outcome—patients will be randomly assigned to endocrine therapy plus chemotherapy. Preliminary results are expected in 2015, but the complete results will not be available for years.

***BluePrint®***

The BluePrint assay identifies breast cancer subtype using an 80-gene assay. It distinguishes luminal from nonluminal subtypes.[50] Patients who are low risk according to MammaPrint are considered Luminal A. Patients who are high risk by MammaPrint and luminal-positive according to BluePrint are considered Luminal B. The BluePrint assay is a microarray-based, proprietary, laboratory-developed test that has not been FDA-cleared. Data suggest that the molecular categories may be used to guide treatment decisions.[51]

***Breast Cancer Index℠ (BCI)***

The Breast Cancer Index (BCI) is a qRT-PCR–based assay that predicts the risk of distant recurrence in patients with hormone receptor–positive, lymph node–negative breast cancer. The test incorporates 2 independent biomarkers: the *HOXB13:IL17BR* ratio and the Molecular Grade Index, a 5-gene panel that consists mostly of proliferation genes.[52] The BCI score, which is generated based on these 2 components, predicts the risk of early recurrence (within 5 years) and late recurrence (after 5 years), as well as the likelihood of benefit from extended endocrine therapy.[13] Like Oncotype DX, the BCI assay is a laboratory-developed test that has not been FDA-cleared. In an evaluation of multiple prognostic assays conducted in a set of 1102 primary tumor samples, the BCI outperformed Oncotype DX in predicting the risk of late recurrence.[53]

Like the Prosigna test, the BCI also identifies the minority of patients who are at risk for late recurrence and thus might be considered as candidates for further extended adjuvant endocrine therapy.[36,53] With the new FDA indication for the Prosigna Assay, patients with a sufficiently low ROR may be able to forego extended adjuvant endocrine therapy. The BCI assay is performed in a central laboratory

using formalin-fixed, paraffin-embedded tissue. The BCI has met the regulatory standards of the Clinical Laboratory Improvement Act (CLIA), but the FDA has chosen not to require regulatory review of this test.[54]

## Considerations When Evaluating Multigene Assays

As a group, the gene expression profiling tests are referred to by the FDA as in vitro diagnostic multivariate index assays (IVDMIAs). These assays take multiple pieces of data, typically in the form of quantitative gene expression, and subject them to an algorithm that yields a specific output that is intended to direct therapy for the patient. Given the potential clinical ramifications of the assays, the FDA considers these tests to be high-impact and high-risk. In an address at the 2013 ASCO meeting, former FDA Commissioner Dr Margaret Hamburg indicated that the FDA plans to exert closer guidance over the development of these tests.[55]

Laboratory-developed tests may claim on their reports that they have been developed in a CLIA-certified laboratory. CLIA refers to federal regulatory standards that apply to all clinical laboratory testing performed on people within the United States. Certification is managed by 3 federal agencies: the FDA, the Center for Medicaid Services, and the Centers for Disease Control. There are no CLIA guidelines for molecular testing. With a lack of gold standards or proficiency guidelines, the claim of CLIA certification for molecular testing holds little weight.

All of these reasons support the desirability of FDA clearance, which would indicate that an independent body has reviewed the data, considered the analytical and clinical validity, and evaluated the clinical utility to some degree before giving a test approval to pass through FDA clearance. Without these procedures in place, patient needs may not be met in the safest possible way. A safety issue of concern is that the parameters for these laboratory-developed tests are known only by the companies that possess them. These companies typically may not have data that reflect the potential risks of the test; for example, rates of false-negative and false-positive results are not known. With FDA clearance, these tests will have documented clinical specificities and sensitivities. Therefore, if an issue develops over time with the test, clinicians and patients will be notified quickly by the FDA (as is done for FDA-approved prescription medicines). In fact, diagnostic device manufacturers are required by FDA regulations to report any adverse events resulting from the use of the device—another safety mechanism not in place for laboratory-developed tests. These added safety measures are a critical piece of today's paradigm of patient-centered personalized medicine.

## Comparing Gene Expression Assays

Several studies have been conducted comparing results obtained with different multigene assays. TransATAC is the only study to include a true comparison of Prosigna vs Oncotype, using remnant RNA from the same patient samples.[9] The 2 tests showed a strong concordance in identifying high-risk and low-risk patients. They differed, however, in that Prosigna identified significantly fewer patients as intermediate risk. Patients who are identified as intermediate risk by Prosigna show a better outcome than those identified by Oncotype.

Kelly and colleagues compared risk assignments based on the PAM50 Assay and the Oncotype DX recurrence score used in the same patient population.[56] Tumor samples were analyzed from 151 patients with ER-positive stage I or II breast cancers. The analysis yielded fairly good agreement between the tests for high-risk and low-risk groups; 90% of high-risk cancers based on the recurrence score were classified as Luminal B, and 1 high-risk tumor was basal-like. Among the low-risk recurrence score tumors, 83% were Luminal A. However, there was a substantial disagreement between the tests among the patients in the intermediate-risk recurrence score group, with half of patients recategorized as low-risk, Luminal A types by the PAM50 test.[56]

Based on these findings, a substantial number of patients would have been treated differently based on which test the oncologist ordered. Half of patients in the intermediate-risk recurrence score category may have received chemotherapy had they undergone Oncotype DX testing, whereas they would not have received chemotherapy based on the PAM50 test. In general, the criticisms that have been leveled at pathologists for their reproducibility of tumor grading have only recently been applied to these molecular gene expression profiling tests, but the findings are not surprising—different tests measuring the expression of different genes and analyzed with different algorithms do have substantial discordances.

Oncologists must work as closely as possible with their pathologists and ensure that test results make sense in the context of the pathology report, which remains the gold standard. The prognostic value of tumor staging, and all the components incorporated into Adjuvant! Online, have been established for 5 decades or longer and will remain the backbone of all that we know about patients with breast cancer and their outcomes. In most cases, results from the test should not vary dramatically from the pathology reports. However, gene expression profiling tests can lead to reclassification of patients based on genomic profiling. As shown in the TransATAC study, a combination of genomic profiling and clinical factors provides the best prognostic information.[53]

In 2014, my colleagues and I presented results of an independent study in which Oncotype DX and MammaPrint analyses were performed on tumor samples from approximately 340 patients with ER-positive tumors with at least 5 years of follow-up from diagnosis.[57] The results showed substantial variation, nearly 30%, in the way risk assignment categories were assigned by the tests. MammaPrint defined 237 cases as low risk; among these patients, the Oncotype recurrence scores were low in 142, intermediate in 91, and high in 4. Among the 107 cases defined as high risk by MammaPrint, recurrence scores were low in 13, intermediate in 43, and high in 51.

Multiple factors may contribute to these discrepancies: the assays use different analytic platforms, are based on different patient populations with varying outcomes, use different gene sets, and have different clinical validity. Between Oncotype DX and MammaPrint, the risk categorization often flips between low- or intermediate-risk Oncotype and low-risk MammaPrint, or between intermediate-risk Oncotype and high-risk MammaPrint.[57] There is no established gold-standard test, and it is difficult to draw conclusions from discordant results.

Additional studies are being undertaken to more comprehensively compare different multigene assays and assess their clinical utility. The OPTIMA (Optimal Personalised Treatment of Early Breast Cancer Using Multi-Parameter Analysis) trial is a randomized, controlled trial being conducted in the United Kingdom that aims to determine the optimal strategy using multigene assays to guide the use of adjuvant therapy in women ages 40 years or older with node-positive, ER-positive, HER2-negative early breast cancer. A preliminary phase (OPTIMA-prelim) is comparing Oncotype DX, MammaPrint, and Prosigna.[58] In the 301 patients evaluated in the OPTIMA-prelim trial, 61% of cases yielded no consensus result among the 3 assays. Investigators concluded that a single most effective test could not be identified, and that cost effectiveness could help triage tests for the main OPTIMA trial. An analysis of the economic impact of the OPTIMA-prelim results have led the investigators to prioritize Prosigna for the main trial.[59]

### *Disclosure*

*Dr Dabbs has no real or apparent conflicts of interest to report.*

## References


1. Rakha EA, El-Sayed ME, Lee AH, et al. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. *J Clin Oncol.* 2008;26(19):3153-3158.
2. van Diest PJ, van der Wall E, Baak JP. Prognostic value of proliferation in invasive breast cancer: a review. *J Clin Pathol.* 2004;57(7):675-681.
3. Slamon D, Eiermann W, Robert N, et al; Breast Cancer International Research Group. Adjuvant trastuzumab in HER2-positive breast cancer. *N Engl J Med.* 2011;365(14):1273-1283.
4. Esteva FJ, Valero V, Booser D, et al. Phase II study of weekly docetaxel and trastuzumab for patients with HER-2-overexpressing metastatic breast cancer. *J Clin Oncol.* 2002;20(7):1800-1808.
5. Fornier M, Esteva FJ, Seidman AD. Trastuzumab in combination with chemotherapy for the treatment of metastatic breast cancer. *Semin Oncol.* 2000;27(6)(suppl 11):38-45.
6. Ravdin PM, Siminoff LA, Davis GJ, et al. Computer program to assist in making decisions about adjuvant therapy for women with early breast cancer. *J Clin Oncol.* 2001;19(4):980-991.
7. Olivotto IA, Bajdik CD, Ravdin PM, et al. Population-based validation of the prognostic model ADJUVANT! for early breast cancer. *J Clin Oncol.* 2005;23(12):2716-2725.
8. Gnant M, Filipits M, Greil R, et al; Austrian Breast and Colorectal Cancer Study Group. Predicting distant recurrence in receptor-positive breast cancer patients with limited clinicopathological risk: using the PAM50 Risk of Recurrence score in 1478 postmenopausal patients of the ABCSG-8 trial treated with adjuvant endocrine therapy alone. *Ann Oncol.* 2014;25(2):339-345.
9. Dowsett M, Sestak I, Lopez-Knowles E, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. *J Clin Oncol.* 2013;31(22):2783-2790.
10. Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol.* 2006;24(23):3726-3734.
11. van't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. *Nature.* 2002;415(6871):530-536.
12. van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. *N Engl J Med.* 2002;347(25):1999-2009.
13. Zhang Y, Schnabel CA, Schroeder BE, et al. Breast cancer index identifies early-stage estrogen receptor-positive breast cancer patients at risk for early- and late-distant recurrence. *Clin Cancer Res.* 2013;19(15):4196-4205.
14. Prat A, Rodriguez BJ, Rovira PS, et al. Blinded independent validation of the PAM50-based Chemo-Endocrine Sensitivity Predictor (CESP) in hormone receptor (HR)-positive/HER2-negative (HR+/HER2-) breast cancer following neoadjuvant chemotherapy (NAC)[ASCO abstract 569]. *J Clin Oncol.* 2015;33(suppl).
15. Chia SK, Bramwell VH, Tu D, et al. A 50-gene intrinsic subtype classifier for prognosis and prediction of benefit from adjuvant tamoxifen. *Clin Cancer Res.* 2012;18(16):4465-4472.
16. Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. *Mol Oncol.* 2011;5(1):5-23.
17. Tutt A, Ellis P, Kilburn L, et al. TNT: a randomized phase III trial of carboplatin (C) compared with docetaxel (D) for patients with metastatic or recurrent locally advanced triple negative or *BRCA1/2* breast cancer (CRUK/07/012). Paper presented at: 37th Annual San Antonio Breast Cancer Symposium; December 9-13, 2014; San Antonio, Texas. Abstract S3-01.
18. Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol.* 2009;27(8):1160-1167.
19. Rodriguez BJ, Fernandez AIL, Ribelles N, et al. Prosigna (PAM50) to predict response to neoadjuvant chemotherapy (NAC) in HR+/HER2- early breast cancer (EBC) patients [ASCO abstract 11049]. *J Clin Oncol.* 2015;33(suppl).
20. Wuerstlein R, Sotlar K, Gluz O, et al. Significance of prospective multicenter decision impact WSG-BCIST Study in postmenopausal ER+ HER2- N0 early breast cancer (EBC) for molecular testing for intrinsic subtype definition [ASCO abstract 535]. *J Clin Oncol.* 2015;33(suppl).
21. Knauer M, Mook S, Rutgers EJ, et al. The predictive value of the 70-gene signature for adjuvant chemotherapy in early breast cancer. *Breast Cancer Res Treat.* 2010;120(3):655-661.
22. Flanagan MB, Dabbs DJ, Brufsky AM, Beriwal S, Bhargava R. Histopathologic variables predict Oncotype DX recurrence score. *Mod Pathol.* 2008;21(10):1255-1261.
23. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature.* 2000;406(6797):747-752.
24. Prosigna Breast Cancer Prognostic Gene Signature Assay [package insert]. Seattle, WA: NanoString Technologies, Inc; 2015.
25. Nielsen T, Wallden B, Schaper C, et al. Analytical validation of the PAM50-based Prosigna Breast Cancer Prognostic Gene Signature Assay and nCounter Analysis System using formalin-fixed paraffin-embedded breast tumor specimens. *BMC Cancer.* 2014;14:177.
26. Reis PP, Waldron L, Goswami RS, et al. mRNA transcript quantification in archival samples using multiplexed, color-coded probes. *BMC Biotechnol.* 2011;11:46.
27. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. *J Natl Cancer Inst.* 2009;101(21)1446-1452.
28. Cheang MCU, Bliss J, Dowsett M, et al. Concordance of intrinsic subtyping and risk of recurrence (ROR) scores between matched primary and metastatic tissue from Triple Negative Breast Cancer Trial (TNT) [ASCO abstract 1019]. *J Clin*

*Oncol*. 2015;33(suppl).
29. Lænkholm A-V, Jensen M-B, Eriksen JO, et al. Prediction of 10yr distant recurrence (DR) using the Prosigna (PAM50) assay in a Danish Breast Cancer Cooperative Group (DBCG) cohort of postmenopausal Danish women with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5yr of endocrine therapy (ET) alone. [ASCO abstract 546]. *J Clin Oncol*. 2015;33(suppl).
30. Ejlertsen B, Jensen M-B, Eriksen JO, et al. Validation of prediction of distant recurrence (DR) by Prosigna (PAM50) in subgroups of a Danish Breast Cancer Cooperative Group (DBCG) cohort of node-positive (N1), hormone receptor positive (HR+), postmenopausal early breast cancer (EBC) patients allocated 5yr of endocrine therapy (ET) [ASCO abstract 513]. *J Clin Oncol*. 2015;33(suppl).
31. Lænkholm A-V, Jensen M-B, Eriksen JO, et al. Prediction of late distant recurrence (DR) using the Prosigna (PAM50) test in a Danish Breast Cancer Cooperative Group (DBCG) cohort of postmenopausal women diagnosed with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5yr of endocrine therapy (ET) [ASCO abstract 544]. *J Clin Oncol*. 2015;33(suppl).
32. Cheang MC, Voduc KD, Tu D, et al. Responsiveness of intrinsic subtypes to adjuvant anthracycline substitution in the NCIC.CTG MA.5 randomized trial. *Clin Cancer Res.* 2012;18(8):2402-2412.
33. Prat A, Bianchini G, Thomas M, et al. Research-based PAM50 subtype predictor identifies higher responses and improved survival outcomes in HER2-positive breast cancer in the NOAH study. *Clin Cancer Res*. 2014;20(2):511-521.
34. Coates AS, Winer EP, Goldhirsch A, et al; Panel members. Tailoring therapies—improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015 [published online May 4, 2015]. *Ann Oncol*. 2015. mdv221.
35. Martín M, Gonzalez-Rivera M, Morales S, et al. Prospective study of the impact of the Prosigna assay on adjuvant clinical decision-making in unselected patients with estrogen receptor positive, human epidermal growth factor receptor negative, node negative early-stage breast cancer. *Curr Med Res Opin*. 2015;31(6):1129-1137.
36. Filipits M, Nielsen TO, Rudas M, et al. The PAM50 risk-of-recurrence score predicts risk for late distant recurrence after endocrine therapy in postmenopausal women with endocrine-responsive early breast cancer. *Clin Cancer Res*. 2014;20(5):1298-1305.
37. Sestak I, Dowsett M, Zabaglo L, et al. Factors predicting late recurrence for estrogen receptor-positive breast cancer. *J Natl Cancer Inst*. 2013;105(19):1504-1511.
38. Sestak I, Cuzick J, Dowsett M, et al. Prediction of late distant recurrence after 5 years of endocrine treatment: a combined analysis of patients from the Austrian breast and colorectal cancer study group 8 and arimidex, tamoxifen alone or in combination randomized trials using the PAM50 risk of recurrence score. *J Clin Oncol*. 2015;33(8):916-922.
39. German Association of Gynecological Oncology. Diagnosis and Treatment of Patients with Primary and Metastatic Breast Cancer. Version 2015.1. http://www.ago-online.de/fileadmin/downloads/leitlinien/mamma/maerz2015/en/2015E_Updated_Guidelines.pdf. Posted March 2015. Accessed June 2, 2015.
40. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med.* 2004;351(27):2817-2826.
41. Ioannidis JP. Is molecular profiling ready for use in clinical decision making? *Oncologist.* 2007;12(3):301-311.
42. Baxter E, Chia SK, Lohrisch CA, et al. Correlation between Nottingham grade and Oncotype DX score in breast cancer: implications for cost-effective incorporation of oncotype in the clinic [Breast Cancer Symposium abstract 106]. *J Clin Oncol.* 2014;32(suppl 26).
43. Dabbs DJ, Klein ME, Mohsin SK, Tubbs RR, Shuai Y, Bhargava R. High false-negative rate of HER2 quantitative reverse transcription polymerase chain reaction of the Oncotype DX test: an independent quality assurance study. *J Clin Oncol*. 2011;29(32):4279-4285.
44. Dvorak L, Dolan M, Fink J, Varghese L, Henriksen J, Gulbahce HE. Correlation between HER2 determined by fluorescence in situ hybridization and reverse transcription-polymerase chain reaction of the oncotype DX test. *Appl Immunohistochem Mol Morphol*. 2013;21(3):196-199.
45. Park MM, Ebel JJ, Zhao W, Zynger DL. ER and PR immunohistochemistry and HER2 FISH versus oncotype DX: implications for breast cancer treatment. *Breast J*. 2014;20(1):37-45.
46. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: breast cancer. Version 2.2015. Updated March 11, 2015. http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Accessed April 14, 2015.
47. Albain KS, Barlow WE, Shak S, et al; Breast Cancer Intergroup of North America. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. *Lancet Oncol*. 2010;11(1):55-65.
48. ClinicalTrials.gov. Hormone therapy with or without combination chemotherapy in treating women who have undergone surgery for node-negative breast cancer (the TAILORx trial). https://clinicaltrials.gov/ct2/show/NCT00310180. ID: NCT00310180. Accessed April 16, 2015.
49. ClinicalTrials.gov. Genetic testing or clinical assessment in determining the need for chemotherapy in women with breast cancer that involves no more than 3 lymph nodes. https://clinicaltrials.gov/ct2/show/NCT00433589. ID: NCT00433589. Accessed April 20, 2015.
50. Glück S, de Snoo F, Peeters J, Stork-Sloots L, Somlo G. Molecular subtyping of early-stage breast cancer identifies a group of patients who do not benefit from neoadjuvant chemotherapy. *Breast Cancer Res Treat*. 2013;139(3):759-767.
51. Whitworth P, Stork-Sloots L, de Snoo FA, et al. Chemosensitivity predicted by BluePrint 80-gene functional subtype and MammaPrint in the Prospective Neoadjuvant Breast Registry Symphony Trial (NBRST). *Ann Surg Oncol*. 2014;21(10):3261-3267.
52. Jerevall PL, Ma XJ, Li H, et al. Prognostic utility of HOXB13:IL17BR and molecular grade index in early-stage breast cancer patients from the Stockholm trial. *Br J Cancer*. 2011;104(11):1762-1769.
53. Sgroi DC, Sestak I, Cuzick J, et al. Prediction of late distant recurrence in estrogen receptor positive breast cancer patients: prospective comparison of the Breast Cancer Index (BCI), Oncotype DX recurrence score, and IHC4 in TransATAC. *Lancet Oncol.* 2013;14(11):1067-1076.
54. Glacy J, Chopra RD. Blue Cross Blue Shield Technology Evaluation Center (TEC) assessment: gene expression profiling in women with lymph node–negative breast cancer to select adjuvant chemotherapy. 2014;29(3). http://www.bcbs.com/blueresources/tec/press/gene-expression-profiling-in.html Publication date: October 2014. Accessed May 26, 2015.
55. Gaffney A. Hamburg: lab-developed tests to be subject to heightened scrutiny under new framework. http://www.raps.org/focus-online/news/news-article-view/article/3551/hamburg-lab-developed-tests-to-be-subject-to-heightened-scrutiny-under-new-fram.aspx. Posted June 3, 2013. Accessed April 28, 2015.
56. Kelly CM, Bernard PS, Krishnamurthy S, et al. Agreement in risk prediction between the 21-gene recurrence score assay (Oncotype DX®) and the PAM50 breast cancer intrinsic Classifier™ in early-stage estrogen receptor-positive breast cancer. *Oncologist*. 2012;17(4):492-498.
57. Dabbs DJ, Brufsky A, Jankowitz RC, et al. Comparison of test results and clinical outcomes of patients assessed with both MammaPrint and Oncotype DX with pathologic variables: an independent study [ASCO abstract 550]. *J Clin Oncol*. 2014;32(suppl 5s).
58. Bartlett JM, Stein RC, Bayani J, et al. Comparison of multiparameter tests in the UK OPTIMA-preliminary trial. [SABCS abstract P4-11-07]. *Cancer Res*. 2015;75(9 suppl).
59. Hall PS, Smith AS, Vargas-Palacios A, et al. UK OPTIMA-prelim study demonstrates economic value in more clinical evaluation of multi-parameter prognostic tests in early breast cancer [SABCS abstract P6-08-11]. *Cancer Res*. 2015;75(9 suppl).

# Genomic Signatures in Breast Cancer: Limitations of Available Predictive Data and the Importance of Prognosis

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**Abstract:** Several biomarkers and gene mutations in breast cancer have been shown to be predictive, in that they determine which treatments a patient should receive. Ideally, predictive markers would be available that could determine the most appropriate treatment plan based on a patient's biology. This goal is becoming a reality in some treatment settings and cancer types, with the increasing use of targeted therapies directed against specific molecular abnormalities. Immunohistochemistry (IHC) testing is in standard use for guiding breast cancer therapy. Testing for the estrogen receptor (ER) and progesterone receptor (PR) guides the use of endocrine therapy, and human epidermal growth factor receptor 2 (HER2) testing guides the use of HER2-targeted therapies. Although IHC provides some discrimination among breast cancer subsets and helps identify appropriate therapy, more information can be gained through gene expression analyses. Contemporary multianalyte assays have demonstrated greater precision and reproducibility than seen with IHC-based assays. The most important contribution of multigene assays is the identification of women with ER/PR-positive, HER2-negative, early-stage breast cancer who are at low risk of recurrence and therefore will likely do well with endocrine therapy alone. These patients can be safely spared from the cytotoxic effects of chemotherapy.

The type of information provided by a biomarker or multigene assay can be categorized as prognostic or predictive. Prognostic markers estimate a patient's clinical outcome, whereas predictive markers estimate a patient's likelihood of benefitting from a specific treatment. It is important to consider this difference when evaluating data and claims for biomarkers or multigene assays. Conventionally, prognosis is considered to be a patient's expected outcome without treatment, thus reflecting the natural history of the disease. However, most studies evaluating the prognostic value of multigene assays have been conducted in patients receiving adjuvant therapy, so they are not purely prognostic in regard to assessing natural history. When evaluating prognostic studies, it is important to consider the treatment history of the patient population. Studies evaluating the association between molecular markers and clinical outcomes in patients receiving adjuvant therapy are considered to be prognostic, not predictive, as they do not assess the likelihood of response to a specific therapy. Data on the predictive value of multigene assays are limited and based on retrospective analyses of prospective studies. A predictive assay that defines which patients are most likely to benefit from a specific systemic chemotherapy would be a valuable tool for optimizing breast cancer therapy.[1]

## Predictive Markers to Determine Appropriate Treatment

Several biomarkers have been shown to be predictive, in that they determine which treatments a patient should receive. An example is human epidermal growth factor receptor 2 (HER2)–positivity, which is clearly predictive of a benefit with HER2-targeted therapy.[2-4] The National Comprehensive Cancer Network guidelines state that the presence of HER2 indicates that HER2-targeted therapy should be administered.[5]

Gene mutations can also serve as predictive markers. In the setting of advanced non–small cell lung cancer, rearrangements in the *ALK* gene predict responses to the ALK inhibitor crizotinib, which has demonstrated significantly greater efficacy compared with chemotherapy.[6] In the setting of advanced ovarian cancer, the presence of germline *BRCA* mutations is associated with responses to

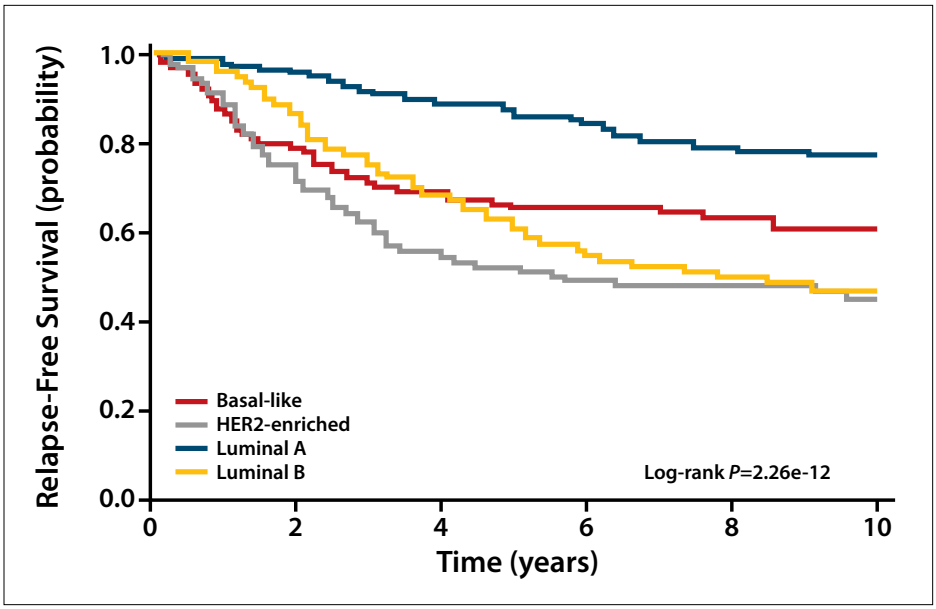


**Figure 1.** Relapse-free survival according to intrinsic subtype among patients who have not received adjuvant systemic therapy. HER2, human epidermal growth factor receptor 2. Adapted from Parker JS et al. *J Clin Oncol*. 2009;27:1160-1167.[20]

olaparib, a poly(ADP-ribose) polymerase inhibitor.[7] Based on its demonstrated efficacy in patients with *BRCA* mutations, olaparib was approved by the US Food and Drug Administration (FDA) for women with heavily pretreated ovarian cancer who have *BRCA* mutations as detected by a specific FDA-approved assay.[8] Olaparib has also demonstrated activity in patients with other *BRCA1/2*-associated advanced cancers, including breast cancer.[9,10]

### *Tailoring Breast Cancer Treatment*

Ideally, predictive markers would be available that could determine the most appropriate treatment plan based on a patient's biology. This goal is becoming a reality in some treatment settings and cancer types, with the increasing use of targeted therapies directed against specific molecular abnormalities. Further elucidation of the molecular biology of different cancers may yield a greater ability to predict outcomes and tailor therapy.[11]

Today, immunohistochemistry (IHC) testing is in standard use for guiding breast cancer therapy. Testing for the estrogen receptor (ER) and progesterone receptor (PR) guides the use of endocrine therapy, and HER2 testing guides the use of HER2-targeted therapies. Patients testing negative for ER, PR, and HER2 (triple-negative breast cancer) receive chemotherapy because no targeted therapies exist for them. Although IHC provides some discrimination among breast cancer subsets and helps identify appropriate therapy, much more information can be gained through gene expression analyses. Numerous studies have demonstrated the variability of IHC-based assay results[12-15] and raised questions about the reproducibility of this platform.[16] Contemporary multianalyte assays have demonstrated greater precision and reproducibility than seen with IHC-based assays[16,17] and may be a preferred choice as new technology becomes more accessible.

Gene expression analyses of breast cancers have identified 4 intrinsic subtypes: Luminal A, Luminal B, HER2-enriched, and basal-like.[18,19] The 50-gene PAM50 gene signature is used to determine intrinsic subtype.[20] The 4 intrinsic subtypes differ in their incidence and survival (Figure 1).[20] This type of characterization at the gene-expression level provides information beyond what can be discerned with IHC marker tests such as ER, PR, and HER2. Studies have shown that these 3 IHC markers do not fully reproduce the intrinsic subtypes.[11,20]

## Current Evidence for Multigene Assays in Early-Stage Breast Cancer

The recurrence score provided by the 21-gene Oncotype DX® is prognostic in patients with ER-positive breast cancer treated with tamoxifen, as it is significantly associated with the risk of distant recurrence.[21,22] The 21-gene recurrence score has also been shown to predict benefit from chemotherapy in women with early-stage breast cancer at high risk of relapse (both node-negative and node-positive).[23,24] The predictive value of the assay is based on a system of risk stratification, in which the recurrence score (0-100) is converted to a risk category (low, intermediate, or high) according to predefined cutoff values. Patients with a recurrence score that falls into the high-risk group are most likely to benefit from adjuvant chemotherapy (Figure 2), whereas patients in the low-risk group gain little, if any, benefit.[23]

Because the predictive value of the recurrence score relies on assigning patients to a risk category, accurate risk stratification is critical. The accuracy of categorization based on the Oncotype DX recurrence score was compared with that based on the Prosigna® (PAM50) risk of recurrence (ROR) score in an analysis of data from the ATAC (Arimidex, Tamoxifen Alone or Combined) trial. Dowsett and colleagues evaluated 1017 patients with ER-positive breast cancer who received endocrine therapy in that trial (Table 1).[25] Compared with the recurrence score, the ROR provided greater differentiation among the risk groups; it classified more patients as high-risk and fewer patients as intermediate-risk. Therefore, concordance between the 2 tests is not high in the intermediate- and high-risk groups. This caveat should be kept in mind when considering the role of the recurrence score in predicting the likelihood of benefit from adjuvant therapy, since a proportion of patients classified as intermediate-risk by this score may in fact be of high enough risk that they would benefit from chemotherapy. A similar study found comparable prognostic utility for the Oncotype DX recurrence score and the Breast Cancer Index® (BCI), although BCI was superior in predicting late distant recurrence.[26] Currently, no assay has been validated to determine specific treatment recommendations for an individual patient.

MammaPrint® is a microarray-based test that provides a prognostic score for breast cancer patients younger than 61 years with stage I/II disease and 0 to 3 positive lymph nodes.[27] It is performed by a central laboratory. MammaPrint measures the mRNA expression of 70 genes and stratifies patients into low-risk or high-risk prognostic groups. This genomic test can discriminate prognosis in the group of patients with ER-positive breast cancers. However, most ER-negative tumors are classified as high risk. Data from a multicenter study (n=541) suggested that only the high-risk ER-positive patients benefited from adjuvant chemotherapy.[28,29] Initially, one of the limitations of the MammaPrint assay was the requirement for fresh tissue. The assay has currently been revised for use in formalin-fixed, paraffin-embedded tissues.[30]

At the 2015 American Society of Clinical Oncology (ASCO) meeting, Cheang and colleagues presented results from a study evaluating the concordance between intrinsic subtypes and ROR groups using tissue samples from patients in the TNT (Triple Negative Breast Cancer) trial.[31,32] Basal-like breast cancer and high-risk ROR scores in primary tumors were substantially conserved with matched positive lymph nodes and recurrent tissue samples. There were no instances in which a primary tumor/non–basal-like breast cancer case was classified as basal-like breast cancer in matched positive lymph nodes or recurrent tissue samples. The Luminal A subtype appeared to change to an aggressive subtype or a higher ROR-defined risk group in matched positive lymph nodes and recurrent tissue samples.

Several studies evaluated the ability of the PAM50 ROR score to predict disease recurrence.[33-35] Three analyses from the Danish Breast Cancer Cooperative Group found that the use of the ROR score improved prediction of distant recurrence. The studies suggested that the ROR score can help identify node-negative[33] and node-positive[34] patients who have an excellent prognosis and can avoid overtreatment with adjuvant chemotherapy and those who will not need endocrine therapy beyond 5 years.[35]

## Predictive Data for Multigene Assays

A series of prospective and retrospective studies have evaluated the predictive role of multigene assays in women with early-stage breast cancer.

### *Predictive Data for the Oncotype DX Assay*

The predictive value of Oncotype DX was evaluated in retrospective analyses of 2 large, prospective trials: the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-20 trial and the Southwestern Oncology Group (SWOG) 8814 trial. NSABP B-20 randomly assigned patients with node-negative, ER-positive breast cancer to tamoxifen alone or tamoxifen with cyclophosphamide, methotrexate, and fluorouracil or methotrexate and fluorouracil.[36] The addition of chemotherapy to tamoxifen was associated with a small improvement in disease-free survival, with 5-year rates of 90% with tamoxifen plus chemotherapy vs 85% with tamoxifen alone ($P$=.01).[36]

In their analysis, Paik and colleagues evaluated whether the Oncotype DX recurrence score predicted benefit from adjuvant chemotherapy in 651 patients from the NSABP B-20 trial.[23,37] In patients with low-risk tumors based on the 21-gene recurrence score, chemotherapy appeared to provide minimal, if any, benefit.[23] Conversely, in patients with high-risk tumors, chemotherapy was associated with a substantial reduction in the risk of 10-year distant recurrence.[23,37] In the NSABP B-20 study, however, it is worth noting that the patients included in the tamoxifen treatment arm analysis were the same patients from which the recurrence score was developed and the cutoffs for the 3 recurrence score groups were defined. Moreover, the adjuvant chemotherapy regimen used in the NSABP B-20 trial—cyclophosphamide, methotrexate, and 5-fluorouracil—was administered concurrently with tamoxifen, which can stop proliferation in endocrine-sensitive tumor cells.[23,37]

The second study evaluating the predictive value of the recurrence score drew data from patients enrolled in SWOG 8814, a phase 3, open-label trial that randomly assigned postmenopausal women with hormone receptor–positive, node-positive breast cancer to cyclophosphamide, doxorubicin, and fluorouracil with concurrent or sequential tamoxifen or tamoxifen alone.[38] Chemotherapy with sequential

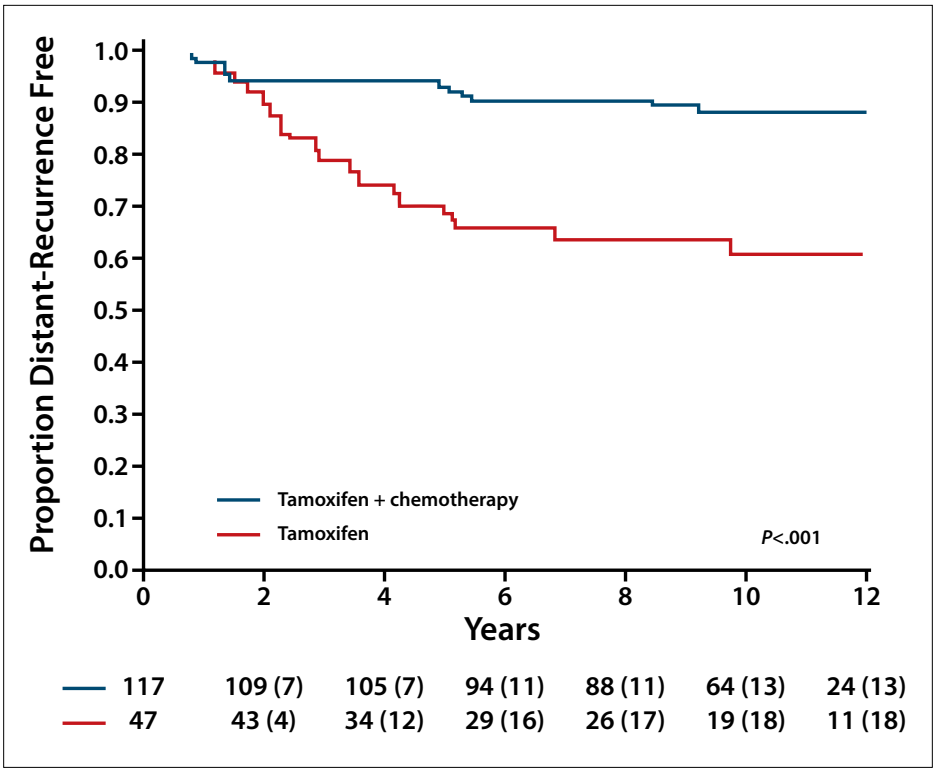


**Figure 2.** Kaplan-Meier plot for distant recurrence comparing treatment with tamoxifen alone vs treatment with tamoxifen plus chemotherapy among high-risk patients. The bottom rows list the number of patients at risk and the number of distant recurrences (in parentheses). Adapted from Paik S et al. *J Clin Oncol.* 2006;24(23):3726-3734.[23]

tamoxifen was associated with a significant improvement in disease-free survival, although the investigators noted that a subset of patients may not benefit from anthracycline-based chemotherapy.[38] In their recurrence score validation study, Albain and colleagues evaluated tumor samples from 367 patients enrolled in SWOG 8814 to determine whether the recurrence score can predict outcomes and benefit from chemotherapy.[24] As in the B-20 study, this analysis found a significant benefit with chemotherapy in patients with a high-risk recurrence score but no benefit in patients with a low-risk score.[24] In both validation studies, the Oncotype DX assay predicted that chemotherapy was beneficial in patients with high-risk tumors. However, the studies were not predictive in the sense that results could be used to identify a single therapy that would be more beneficial in individual patients.

A strength of both retrospective analyses is their use of data from a prospective, randomized trial of endocrine therapy with or without chemotherapy. Few studies have been conducted in this type of patient population. Additionally, the long follow-up in both trials enabled calculation of distant recurrence rates at 10 years.[23,24]

A limitation of these studies is their relatively small size. The analysis of B-20 included 28% of patients enrolled in the study, and the analysis of SWOG 8814 included 40% of the enrolled patients.[23,24] In the B-20 analysis, there were 353 patients at low risk, 134 at intermediate risk, and 164 at high risk. The SWOG 8814 analysis included 146 patients at low risk, 103 at intermediate risk, and 118 at high risk.

The study designs of the original trials might have biased the results. SWOG 8814 included only postmenopausal women, whereas B-20 included both premenopausal and postmenopausal patients.[23,24] Another aspect that may have influenced the results was the inclusion of some patients in both the "test set" and the "validation set," which would not be acceptable today with the development of modern genomic prognostic or predictive assays.[39]

These analyses are also limited by their retrospective nature. Since the design of these trials, guidelines have been proposed to maximize the likelihood that studies using archived specimens provide reliable assessments of the clinical validity of a prognostic or predictive marker.[40] These recommendations include using adequate amounts of archived tissue to attain statistical power, analytically validating the assay for use with a defined classifier, and validating study results using specimens from at least one similar but separate study.[40]

Although these steps can maximize the reliability of biomarker studies, a prospective, randomized trial evaluating the utility of a biomarker is the gold standard.[40] Two

**Table 1.** Comparison of Proportion of Node-Negative Patients by Risk Group Using Risk of Recurrence Plus Tumor Size Vs Recurrence Score

| | Low | | Intermediate | | High | | Total | |
|---|---|---|---|---|---|---|---|---|
| **Test** | **Number** | **%** | **Number** | **%** | **Number** | **%** | **Number** | **%** |
| ROR (including tumor size) | | | | | | | | |
| No CTS | | | | | | | | |
| RS | | | | | | | | |
| Low | 318 | | 97 | | 19 | | 434 | 58.7 |
| Intermediate | 113 | | 68 | | 62 | | 243 | 32.9 |
| High | 6 | | 14 | | 42 | | 62 | 8.4 |
| Total | 437 | 59.1 | 179 | 24.2 | 123 | 16.6 | 739 | 100 |
| CTS included | | | | | | | | |
| RS | | | | | | | | |
| Low | 386 | | 53 | | 1 | | 440 | 59.5 |
| Intermediate | 61 | | 94 | | 46 | | 201 | 27.2 |
| High | 4 | | 18 | | 76 | | 98 | 13.3 |
| Total | 451 | 61.0 | 165 | 22.3 | 123 | 16.6 | 739 | 100 |

CTS, clinical treatment score; ROR, risk of recurrence; RS, recurrence score.

Data from Dowsett M et al. *J Clin Oncol.* 2013;31(22):2783-2790.[25]

such prospective, randomized trials have been undertaken to further evaluate the role of the Oncotype DX assay for predicting responses to chemotherapy. The RxPONDER (Rx for Positive Node, Endocrine Responsive Breast Cancer) trial (SWOG S1007) is prospectively assessing the role of chemotherapy in patients with node-positive disease and a low-risk or intermediate-risk recurrence score.[41] The TAILORx (Trial Assigning Individualized Options for Treatment [Rx]) trial is evaluating the effect of chemotherapy in patients with a midrange recurrence score.[42] The RxPONDER and TAILORx are both large trials, enrolling 4000 and 11,248 patients, respectively. Results from these trials will provide further information on the value of Oncotype DX for predicting response to adjuvant breast cancer chemotherapy. The OPTIMA (Optimal Personalised Treatment of Early Breast Cancer Using Multi-Parameter Analysis) trial is evaluating the role of adjuvant chemotherapy in patients with node-positive, ER-positive, and HER2-negative disease. A preliminary phase is assessing several multigene assays (including Oncotype DX and Prosigna [PAM50]) in approximately 300 patients to determine what would be the best test for patient selection in the main trial (n=3000).[43] An economic analysis of the OPTIMA-prelim results showed Prosigna to be the assay of choice.[44]

### *Predictive Data for the Prosigna (PAM50) Assay*

Guidelines from the German Association of Gynecological Oncology, updated in March 2015, have added use of Prosigna for newly diagnosed patients with node-negative or node-positive, HR-positive, HER2-negative, early-stage breast cancer without clinicopathologic factors that indicate a clear therapeutic decision.[45] Several studies have suggested that PAM50 can predict response to chemotherapy.[32,46-48] For example, Prat and colleagues found that the PAM50 proliferation signature was significantly predictive of responses to chemotherapy and extended survival after chemotherapy in patients with the basal-like subtype.[46] In an analysis of the National Cancer Institute of Canada Clinical Trials Group MA.12 study, Chia and coworkers found that luminal subtypes predicted whether a patient would benefit from tamoxifen.[47] Prat and associates found that trastuzumab-based chemotherapy was especially beneficial in women with advanced HER2-positive/HER2-enriched tumors and HER2-positive tumors that were predicted to have a high risk of relapse and proliferation status as identified by the PAM50 Assay.[48] HER2-enriched tumors had a better response to trastuzumab. In a study by Tutt and colleagues, triple-negative breast cancer patients with a non-basal subtype were more likely to respond to docetaxel than carboplatin.[32] Parker and colleagues, who developed the PAM50 gene set, indicated the significance of the intrinsic subtype identified by PAM50 in both treatment-naive patients and those receiving neoadjuvant chemotherapy.[20] A study presented at the 2015 ASCO meeting by Rodriguez and colleagues also provided predictive data for the Prosigna (PAM50) Assay.[49] In this evaluation, the Prosigna ROR score significantly predicted response to neoadjuvant chemotherapy.[49] Patients with Luminal A tumors were the least likely to respond to neoadjuvant chemotherapy as compared

with the other intrinsic subtypes, which supports the current St Gallen guidelines, updated in May 2015.[50] The intrinsic subtype as identified by the assay showed significant prognostic value in a multivariate analysis accounting for ER status, histologic grade, tumor size, and nodal status.[20]

***Predictive Data for the MammaPrint DX Assay***

The MammaPrint test was developed as a prognostic assay. Retrospective studies showed discordant results for risk stratification using the MammaPrint results and clinical prognostic information together.[51,52] When both MammaPrint and clinical assessment were consistent with either low or high ROR, there was confidence in the prognosis assessment. However, when the MammaPrint was low risk and the clinical information suggested high risk, or vice versa, it was not clear which was more predictive. A prospective randomized trial (MINDACT [Microarray in Node Negative and 1-3 Positive Lymph Node Disease May Avoid Chemotherapy]) was launched and completed to determine the prognostic and predictive roles of MammaPrint in patients with early-stage ER/PR-positive breast cancer with discordant prognostic assessment by clinical data and assigned MammaPrint risk group.[53] These patients were randomized to adjuvant chemotherapy followed by endocrine therapy vs endocrine therapy alone. The results are awaited and should help determine whether the MammaPrint test can predict which patients are likely to benefit from adjuvant chemotherapy.

## Class Effect

Prognostic multigene assays that accurately assess ROR based on biology can provide a similar level of prediction of chemotherapy benefit as the Oncotype DX test. This class effect relates to the tests' overlap in key biological pathways, such as the ER, HER2, and proliferation-related genes. Therefore, patients with tumors that have high ER expression, low HER2 expression, and low proliferation results are likely to be categorized as low risk regardless of the test. Conversely, patients with low ER or PR expression, high HER2 expression, and a high proliferation index will have high-risk disease. The 2015 guidelines from the St Gallen International Expert Consensus support the concept of a class effect for multigene assays because prognostic tests that are not specifically predictive of the efficacy of cytotoxic therapy are commonly used to make decisions about such therapy.[50] This approach is justified because these tests likely define a group of patients with a prognosis so good that even if chemotherapy were similarly proportionately effective as in higher risk patients, the absolute benefit may be thought insufficient to justify such treatment. Similarly, a test result indicating a worse prognosis may be used to justify the use of effective but more toxic endocrine therapy, such as ovarian function suppression plus aromatase inhibitors or more intensive or prolonged chemotherapy. In this context, Oncotype DX, MammaPrint, Prosigna (PAM50), and BCI each appears to identify a group of patients for whom the risk of distant metastases is so low that the benefit of adjuvant chemotherapy would be outweighed by the risks.

## Conclusion

The most important contribution of multigene assays is the identification of women with ER/PR-positive, HER2-negative, early-stage breast cancer who are at low ROR and therefore will likely do well with endocrine therapy alone. Those patients can be safely spared from the cytotoxic effects of chemotherapy. In contrast, women whose tumors are categorized as high risk using Oncotype DX, Prosigna (PAM50), MammaPrint, or BCI should be considered for adjuvant chemotherapy. Therefore, until data from prospective, randomized trials are available, adjuvant chemotherapy should be discussed with all women at intermediate ROR in the context of other prognostic and clinical factors.

***Disclosure***

*Dr Esteva is a consultant for NanoString Technologies, Inc., which offers the Prosigna® Breast Cancer Prognostic Gene Signature Assay, based on the PAM50 gene signature.*

## References

1. Adaniel C, Jhaveri K, Heguy A, Esteva FJ. Genome-based risk prediction for early stage breast cancer. *Oncologist*. 2014;19(10):1019-1027.
2. Slamon D, Eiermann W, Robert N, et al; Breast Cancer International Research Group. Adjuvant trastuzumab in HER2-positive breast cancer. *N Engl J Med.* 2011;365(14):1273-1283.
3. Esteva FJ, Valero V, Booser D, et al. Phase II study of weekly docetaxel and trastuzumab for patients with HER-2-overexpressing metastatic breast cancer. *J Clin Oncol.* 2002;20(7):1800-1808.
4. Fornier M, Esteva FJ, Seidman AD. Trastuzumab in combination with chemotherapy for the treatment of metastatic breast cancer. *Semin Oncol.* 2000;27(6)(suppl 11):38-45.
5. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology: breast cancer. Version 2.2015. Updated March 11, 2015. http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf. Accessed April 14, 2015.
6. Solomon BJ, Mok T, Kim DW, et al; PROFILE 1014 Investigators. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. *N Engl J Med.* 2014;371(23):2167-2177.
7. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol.* 2014;15(8):852-861.
8. FDA approves Lynparza to treat advanced ovarian cancer. US Food and Drug Administration. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm427554.htm. Posted December 19, 2014. Accessed May 29, 2015.
9. Tutt A, Robson M, Garber JE, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. *Lancet.* 2010;376(9737):235-244.
10. Kaufman B, Shapira-Frommer R, Schmutzler RK, et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. *J Clin Oncol.* 2015;33(3):244-250.
11. Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. *Mol Oncol.* 2011;5(1):5-23.

12. Seidenfeld J, Samson DJ, Rothenberg BM, et al. HER2 Testing to Manage Patients With Breast Cancer or Other Solid Tumors. Evidence Reports/Technology Assessments, No. 172. Rockville (MD): Agency for Healthcare Research and Quality (US); 2008 Nov. http://www.ncbi.nlm.nih.gov/books/NBK38617/]
13. Ross JS, Slodkowska EA, Symmans WF, Pusztai L, Ravdin PM, Hortobagyi GN. The HER-2 receptor and breast cancer: ten years of targeted anti-HER-2 therapy and personalized medicine. *Oncologist*. 2009;14(4):320-368.
14. Zaha DC. Significance of immunohistochemistry in breast cancer. *World J Clin Oncol*. 2014;5(3):382-392.
15. Potts SJ, Krueger JS, Landis ND, et al. Evaluating tumor heterogeneity in immunohistochemistry-stained breast cancer tissue. *Lab Invest*. 2012;92(9):1342-1357.
16. Hefti MM, Hu R, Knoblauch NW, et al. Estrogen receptor negative/progesterone receptor positive breast cancer is not a reproducible subtype. *Breast Cancer Res*. 2013;15(4):R68.
17. Bastien RR, Rodríguez-Lescure Á, Ebbert MT, et al. PAM50 breast cancer subtyping by RT-qPCR and concordance with standard clinical molecular markers. *BMC Med Genomics*. 2012;5:44.
18. Perou CM, Sørlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature*. 2000;406(6797):747-752.
19. Sørlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A*. 2001;98(19):10869-10874.
20. Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol*. 2009;27(8):1160-1167.
21. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*. 2004;351(27):2817-2826.
22. Dowsett M, Cuzick J, Wale C, et al. Prediction of risk of distant recurrence using the 21-gene recurrence score in node-negative and node-positive postmenopausal patients with breast cancer treated with anastrozole or tamoxifen: a TransATAC study. *J Clin Oncol*. 2010;28(11):1829-1834.
23. Paik S, Tang G, Shak S, et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol*. 2006;24(23):3726-3734.
24. Albain KS, Barlow WE, Shak S, et al; Breast Cancer Intergroup of North America. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. *Lancet Oncol*. 2010;11(1):55-65.
25. Dowsett M, Sestak I, Lopez-Knowles E, et al. Comparison of PAM50 risk of recurrence score with oncotype DX and IHC4 for predicting risk of distant recurrence after endocrine therapy. *J Clin Oncol*. 2013;31(22):2783-2790.
26. Sgroi DC, Sestak I, Cuzick J, et al. Prediction of late distant recurrence in patients with oestrogen-receptor-positive breast cancer: a prospective comparison of the breast-cancer index (BCI) assay, 21-gene recurrence score, and IHC4 in the TransATAC study population. *Lancet Oncol*. 2013;14(11):1067-1076.
27. Mook S, Schmidt MK, Viale G, et al; TRANSBIG Consortium. The 70-gene prognosis-signature predicts disease outcome in breast cancer patients with 1-3 positive lymph nodes in an independent validation study. *Breast Cancer Res Treat*. 2009;116(2):295-302.
28. Knauer M, Mook S, Rutgers EJ, et al. The predictive value of the 70-gene signature for adjuvant chemotherapy in early breast cancer. *Breast Cancer Res Treat*. 2010;120(3):655-661.
29. Drukker CA, Bueno-de-Mesquita JM, Retèl VP, et al. A prospective evaluation of a breast cancer prognosis signature in the observational RASTER study. *Int J Cancer*. 2013;133(4):929-936.
30. Mittempergher L, de Ronde JJ, Nieuwland M, et al. Gene expression profiles from formalin fixed paraffin embedded breast cancer tissue are largely comparable to fresh frozen matched tissue. *PLoS One*. 2011;6(2):e17163. doi: 10.1371/journal.pone.0017163
31. Cheang MCU, Bliss J, Dowsett M, et al. Concordance of intrinsic subtyping and risk of recurrence (ROR) scores between matched primary and metastatic tissue from Triple Negative Breast Cancer Trial (TNT) [ASCO abstract 1019]. *J Clin Oncol*. 2015;33(suppl).
32. Tutt A, Ellis P, Kilburn L, et al. TNT: a randomized phase III trial of carboplatin (C) compared with docetaxel (D) for patients with metastatic or recurrent locally advanced triple negative or *BRCA1/2* breast cancer (CRUK/07/012). Paper presented at: 37th Annual San Antonio Breast Cancer Symposium; December 9-13, 2014; San Antonio, Texas. Abstract S3-01.
33. Lænkholm A-V, Jensen M-B, Eriksen JO, et al. Prediction of 10yr distant recurrence (DR) using the Prosigna (PAM50) assay in a Danish Breast Cancer Cooperative Group (DBCG) cohort of postmenopausal Danish women with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5yr of endocrine therapy (ET) alone [ASCO abstract 546]. *J Clin Oncol*. 2015;33(suppl).
34. Ejlertsen B, Jensen M-B, Eriksen JO, et al. Validation of prediction of distant recurrence (DR) by Prosigna (PAM50) in subgroups of a Danish Breast Cancer Cooperative Group (DBCG) cohort of node-positive (N1), hormone receptor positive (HR+), postmenopausal early breast cancer (EBC) patients allocated 5yr of endocrine therapy (ET) [ASCO abstract 513]. *J Clin Oncol*. 2015;33(suppl).
35. Lænkholm A-V, Jensen M-B, Eriksen JO, et al. Prediction of late distant recurrence (DR) using the Prosigna (PAM50) test in a Danish Breast Cancer Cooperative Group (DBCG) cohort of postmenopausal women diagnosed with hormone receptor-positive (HR+) early breast cancer (EBC) allocated to 5yr of endocrine therapy (ET) [ASCO abstract 544]. *J Clin Oncol*. 2015;33(suppl).
36. Fisher B, Dignam J, Wolmark N, et al. Tamoxifen and chemotherapy for lymph node-negative, estrogen receptor-positive breast cancer. *J Natl Cancer Inst*. 1997;89(22):1673-1682.
37. Paik S. Development and clinical utility of a 21-gene recurrence score prognostic assay in patients with early breast cancer treated with tamoxifen. *Oncologist*. 2007;12(6):631-635.
38. Albain KS, Barlow WE, Ravdin PM, et al; Breast Cancer Intergroup of North America. Adjuvant chemotherapy and timing of tamoxifen in postmenopausal patients with endocrine-responsive, node-positive breast cancer: a phase 3, open-label, randomised controlled trial. *Lancet*. 2009;374(9707):2055-2063.
39. Kos Z, Nielsen TO. Developing a new generation of breast cancer clinical gene expression tests. *Breast Cancer Res.* 2014;16(4):103. doi: 10.1186/bcr3688.
40. Simon RM, Paik S, Hayes DF. Use of archived specimens in evaluation of prognostic and predictive biomarkers. *J Natl Cancer Inst*. 2009;101(21):1446-1452.
41. ClinicalTrials.gov. Tamoxifen citrate, letrozole, anastrozole, or exemestane with or without chemotherapy in treating patients with invasive RxPONDER breast cancer. ID: NCT01272037. https://clinicaltrials.gov/ct2/show/NCT01272037. Accessed April 16, 2015.
42. ClinicalTrials.gov. Hormone therapy with or without combination chemotherapy in treating women who have undergone surgery for node-negative breast cancer (the TAILORx trial). https://clinicaltrials.gov/ct2/show/NCT00310180. ID: NCT00310180. Accessed April 16, 2015.
43. Bartlett JM, Stein RC, Bayani J, et al. Comparison of multiparameter tests in the UK OPTIMA-preliminary trial. [SABCS abstract P4-11-07]. *Cancer Res*. 2015;75(9 suppl).
44. Hall PS, Smith AS, Vargas-Palacios A, et al. UK OPTIMA-prelim study demonstrates economic value in more clinical evaluation of multi-parameter prognostic tests in early breast cancer [SABCS abstract P6-08-11]. *Cancer Res*. 2015;75(9 suppl).
45. German Association of Gynecological Oncology. Diagnosis and Treatment of Patients with Primary and Metastatic Breast Cancer. Version 2015.1. http://www.ago-online.de/fileadmin/downloads/leitlinien/mamma/maerz2015/en/2015E_Updated_Guidelines.pdf. Posted March 2015. Accessed June 2, 2015.
46. Prat A, Lluch A, Albanell J, et al. Predicting response and survival in chemotherapy-treated triple-negative breast cancer. *Br J Cancer*. 2014;111(8):1532-1541.
47. Chia SK, Bramwell VH, Tu D, et al. A 50-gene intrinsic subtype classifier for prognosis and prediction of benefit from adjuvant tamoxifen. *Clin Cancer Res.* 2012;18(16):4465-4472.
48. Prat A, Bianchini G, Thomas M, et al. Research-based PAM50 subtype predictor identifies higher responses and improved survival outcomes in HER2-positive breast cancer in the NOAH study. *Clin Cancer Res*. 2014;20(2):511-521.
49. Rodriguez BJ, Fernandez AIL, Ribelles N, et al. Prosigna (PAM50) to predict response to neoadjuvant chemotherapy (NAC) in HR+/HER2- early breast cancer (EBC) patients [ASCO abstract 11049]. *J Clin Oncol*. 2015;33(suppl).
50. Coates AS, Winer EP, Goldhirsch A, et al; Panel members. Tailoring therapies—improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015 [published online May 4, 2015]. *Ann Oncol*. 2015. mdv221.
51. Mook S, Van't Veer LJ, Rutgers EJ, Piccart-Gebhart MJ, Cardoso F. Individualization of therapy using Mammaprint: from development to the MINDACT Trial. *Cancer Genomics Proteomics*. 2007;4(3):147-155.
52. Bogaerts J, Cardoso F, Buyse M, et al; TRANSBIG consortium. Gene signature evaluation as a prognostic tool: challenges in the design of the MINDACT trial. *Nat Clin Pract Oncol*. 2006;3(10):540-551.
53. ClinicalTrials.gov. Genetic testing or clinical assessment in determining the need for chemotherapy in women with breast cancer that involves no more than 3 lymph nodes. https://clinicaltrials.gov/ct2/show/NCT00433589. ID: NCT00433589. Accessed April 20, 2015.


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