Clinical application and utility of genomic assays in early-stage breast cancer: key lessons learned to date

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ABSTRACT

Early-stage hormone receptor–positive breast cancer is the most common subtype and stage presenting in countries with organized screening programs. Standard clinical and pathologic factors are routinely used to support prognosis and decisions about adjuvant therapies. Hormone receptor and HER2 status are essential for decision-making about the use of adjuvant hormonal and anti-HER2 therapies respectively. Genomic assays are now commercially available to aid in either further prognostication or in refining the potential benefit of adjuvant chemotherapy. The current genomic assays all generally quantify estrogen receptor and proliferation gene sets (among others) by RNA expression, although the specific genes assayed are quite discordant. The present review focuses on the pivotal studies in which each assay attempted to demonstrate clinical utility, with an emphasis on prospective trial data for each assay, if available. Using genomic assays, health care providers will increasingly be able to individualize therapy or de-escalate therapy, optimizing clinic benefit while minimizing toxicities from systemic therapies.

Key Words Breast cancer, genomic assays

INTRODUCTION

Clinical decision-making about adjuvant locoregional and systemic therapies has historically been based on baseline patient- and tumour-related prognostic factors. Patient age, comorbidities, tumour size, tumour grade, lymphovascular invasion, and nodal status have been the primary prognostic factors routinely considered. Only the predictive factors of estrogen receptor (ER) and HER2 (human epidermal growth factor receptor 2) status are clinically useful and validated for, respectively, adjuvant hormonal and anti-HER2–directed therapy. What is clear, however, is that overtreatment occurs in many cases of early-stage breast cancer (EBC). In a population-based study from British Columbia, most of the 1187 T1–2N0 (ER-positive and -negative) EBC patients not treated with adjuvant systemic therapies (>70%) did not recur locoregionally or distant within 10 years of diagnosis1.

In an early-stage EBC consultation, the most difficult and challenging clinical decision is perhaps the recommendation for or against adjuvant chemotherapy and the anticipated benefit to the individual. Based on the individual patient meta-analysis from the Early Breast Cancer Trialists' Collaborative Group, the absolute benefit from adjuvant chemotherapy is modest, particularly in ER-positive EBC2. The hope is that using a genomic assay to reach a more in-depth and quantitative assessment of the biology of an individual’s tumour could provide added prognostic and, in particular, predictive accuracy for the selection of adjuvant systemic therapies. In fact, that hope has in part been realized with the demonstration that, in real-world data, use of the 21-gene recurrence score (nS) assay (OncoType dx: Genomic Health, Redwood, CA, U.S.A.), has led to significant alterations in the use of chemotherapy by age group3.
TABLE I  Currently available genomic assays in estrogen receptor–positive early-stage breast cancer

| Assay            | Classifiers (n genes) | Platform | Binary (high vs. low) | Decentralized testing | Recommended by ASCO clinical practice guideline\(^5\) (node-negative) | Validated in N0 and N1 | Utility in late recurrence |
|------------------|-----------------------|----------|-----------------------|-----------------------|--------------------------------------------------------------------|------------------------|---------------------------|
| Oncotype DX\(^a\) | 16                    | qPCR     | No                    | No                    | Evidence quality: high Strength recommendation: strong              | Yes                    | Possibly                   |
| Prosigna\(^b\)   | 50                    | nCounter | Yes                   | No                    | Evidence quality: high Strength recommendation: strong              | Yes                    | Yes                       |
| MammaPrint\(^c\) | 70                    | Microarray or qPCR | Yes | No | Evidence quality: intermediate Strength recommendation: moderate | Yes | No |
| EndoPredict\(^d\) | 8                     | qPCR     | Yes                   | Yes                   | Evidence quality: intermediate Strength recommendation: moderate | Yes | Possibly |
| Breast Cancer Index\(^e\) | 7 | qPCR     | Yes                   | No                    | Evidence quality: intermediate Strength recommendation: moderate | No | Yes |
| Genomic Grade Index\(^f\) | 97 | Microarray | Yes | No | Not discussed | No | No |

\(^a\) Genomic Health, Redwood City, CA, U.S.A.
\(^b\) NanoString Technologies, Seattle, WA, U.S.A.
\(^c\) Agendia, Irvine, CA, U.S.A.
\(^d\) Myriad Genetics, Salt Lake City, U.S.A.
\(^e\) bioTheranostics, San Diego, CA, U.S.A.
\(^f\) MapQuant Dx: Ipsogen, Marseille, France.

In general, the gene sets within each of the assays are the estrogen-responsive genes, the HER2 pathway–associated genes, and a number of proliferation genes plus a number of housekeeping genes. Interestingly, the overlap of genes between the assays is minimal, with no gene set in Oncotype dx, Prosigna, and MammaPrint (Agendia, Irvine, CA, U.S.A.) being identical. Oncotype dx and Prosigna are the most similar, having 11 genes in common. MammaPrint and Prosigna share 3 genes, and Oncotype dx and MammaPrint have only 1 gene in common. These genomic assays have been investigated predominantly in the estrogen-positive and node-negative (stages I–II) setting for prognosis and, in some instances, prediction.

The present review focuses on the landmark foundation studies in which each assay attempted to demonstrate clinical utility, with a focus on prospective trial data (if available) for each assay (summarized in Table I).

**Oncotype DX**

Oncotype dx is a high-throughput, real-time, reverse transcriptase polymerase chain reaction assay that quantifies gene expression in formalin-fixed paraffin-embedded material (FFPE). A panel of 16 cancer-related genes and 5 reference genes was selected based on its ability to provide prognostic information using tamoxifen-only treated training sets. The 21 genes were then applied to FFPE tumour samples collected during a large multicentre National Surgical Adjuvant Breast and Bowel Project (NSABP) trial (NSABP B-14) in patients with node-negative estrogen-positive early-bca treated with tamoxifen alone. The aim was to quantify the risk of a distant recurrence both as a categorical score (low, intermediate, and high risk) and as a continuous variable (ns: 0–100)\(^11\). The 10-year distant recurrence rates were 6.8% (95% confidence interval (CI): 4.0% to 9.6%) in the low-risk group, 14.3% (95% CI: 8.3% to 20.3%) in the intermediate-risk group, and 30.5% (95% CI: 23.6% to 37.4%) in the high-risk group.

A recent study based on the U.S. Surveillance, Epidemiology, and End Results Program database (38,568 patients, 40–84 years of age, with node-negative and estrogen-positive, HER2-negative early breast cancer and an Oncotype dx result) supported an independent prognostic effect of the genomic assay—beyond standard clinical and pathologic factors—for predicting 5-year breast-cancer-specific mortality\(^4\). The unadjusted 5-year breast-cancer-specific mortality rates were 0.4% (95% CI: 0.3% to 0.6%) for a NS of less than 18, 1.4% (95% CI: 1.1% to 1.7%) for a NS of 18–30, and 4.4% (95% CI: 3.4% to 5.6%) for a NS of 31 or greater.

More clinically relevant, however, was the application of the 21-gene assay to material collected from the nsabp-B-20 trial\(^12\). That landmark trial randomly assigned 2363 node-negative (N0) estrogen-positive breast cancer patients to tamoxifen alone, to tamoxifen and concurrent cyclophosphamide–methotrexate–fluorouracil chemotherapy, or to tamoxifen and concurrent methotrexate–fluorouracil adjuvant chemotherapy. Tumour samples from 651 patients were available for Oncotype dx testing. Patients having tumours with a high score (≥31) experienced a large benefit from chemotherapy (relative risk: 0.26; 95% CI: 0.13 to 0.53; absolute mean decrease in 10-year distant recurrence rate: 27.6%). Patients with tumours having a low score (<18) experienced a negligible benefit from chemotherapy (relative risk: 1.31; 95% CI: 0.46 to 3.78; absolute decrease in distant recurrence rate at 10 years: –1.1% ± 2.2%). Patients with tumours having an intermediate score did not appear to experience a large benefit from chemotherapy, although an absolute benefit of 5% could not be excluded. The test for interaction between chemotherapy treatment and ns was statistically significant (p = 0.038). The results of the study demonstrated that the RFS has an ability to predict the magnitude of the benefit associated with adjuvant chemotherapy.

Lastly, a prospective validation for Oncotype dx was obtained during the tailorx trial (see NCT00310180 at
**TABLE II**  Pivotal studies in which genomic assays have been evaluated for clinical utility

| Assay                        | Pivotal study or studies                  | Study design                    | Sample size (n) | Intervention                                | Clinical utility                                                                 |
|------------------------------|------------------------------------------|----------------------------------|-----------------|---------------------------------------------|----------------------------------------------------------------------------------|
| Oncotype DX                  | NSABP B20                                 | Prospective-retrospective        | 651             | Tamoxifen ± CMF                             | **Significant benefit to chemotherapy when recurrence score is high; limited benefit when recurrence score is low** |
|                              | TAILORx                                   | Prospective                      | 1626            | Endocrine for 5 years                      | **Very favourable prognosis with endocrine therapy alone when recurrence score is 10 or less** |
| Prosigna^a                   | ABCSG-8 and TransATAC^b                   | Prospective-retrospective        | 2137            | Endocrine for 5 years                      | **Very favourable prognosis with endocrine therapy alone when risk-of-recurrence score is low or subtype is luminal A** |
|                              | DBCG^c                                    | Retrospective                    | 2749            | Endocrine for 5 years                      |                                                                                  |
| MammaPrint^c                 | MINDACT^b                                 | Prospective randomized controlled trial | 6693 (entire study) | 2142 (randomized component)                | **Discordance in clinical and genomic results randomized to chemotherapy or not** |
|                              |                                         |                                  |                 |                                             | **Favourable prognosis with or without adjuvant chemotherapy when 70-gene signature is low-risk** |
| EndoPredict^d                | ABCSG-6 and ABCSG-8^a                     | Prospective-retrospective        | 1702            | Endocrine for 5 years                      | **Very favourable prognosis with endocrine therapy alone when EPclin score is low** |
| Breast Cancer Index^e        | CCTG MA.17                                | Nested case-control study        | 249             | Letrozole vs. placebo after 5 years of tamoxifen | **Greater benefit to extended hormonal therapy when the Breast Cancer Index is high** |

CMF = cyclophosphamide, methotrexate, 5-fluorouracil; ROR = risk of recurrence; ABCSG = Austrian Breast and Colorectal Cancer Study Group; DBCG = Danish Breast Cancer Group; CCTG = Canadian Cancer Trials Group.

^a Genomic Health, Redwood City, CA, U.S.A.

^b NanoString Technologies, Seattle, WA, U.S.A.

^c Agendia, Irvine, CA, U.S.A.

^d Myriad Genetics, Salt Lake City, U.S.A.

^e biotheranostics, San Diego, CA, U.S.A.

http://ClinicalTrials.gov), demonstrating strong prognostic discrimination for a rs of 10 or less. Within the larger TAILORx trial, a prospective cohort study looked at patients with tumours having an Oncotype nx rs of 10 or less. All patients were locally assessed as ER-positive and HER2-negative, with no lymph nodes involved (N0). The systemic treatment recommended for this cohort of 1626 women (among the 10,253 eligible women in the TAILORx trial) was adjuvant hormonal therapy alone (no chemotherapy). In that cohort, 70% were postmenopausal, 68% had undergone breast-conserving surgery, median tumour size was 1.74 ± 0.77 cm, one third were locally assessed as grade 1, and 7% were locally assessed as grade 3. Median follow-up in this low-risk cohort was 69 months. A 5-year relapse-free interval was reached in 98.7% (95% CI: 97.9% to 99.2%), the 5-year distant recurrence-free survival was 99.3% (95% CI: 98.7% to 99.6%), and the 5-year overall survival rate was 98% (95% CI: 97.1% to 98.6%; Table II). This cohort within TAILORx will clearly benefit with longer follow-up, but currently shows that, for patients who fit into the low-risk category, treatment with hormonal therapy alone is associated with excellent 5-year clinical outcomes, thus validating the original prognostic discrimination associated with Oncotype nx based on the tamoxifen-treated arm of the NSABP B-14 trial.

**Prosigna**

One of the first gene expression profiling studies in bca is high by Sorlie and colleagues^13 identified 4 distinct molecular subtypes of bca, termed luminal A, luminal B, basal-like, and HER2-enriched. The use of the term “luminal” for bca has generated biologic relevance to clinically distinguishing between higher-risk and lower-risk hormone receptor–positive bca, although in the strictest terms, “luminal” should be used in conjunction with gene expression profiling segregation. Prosigna is a commercialized assay that can use FFPE material to identify a subtype based on Pearson correlation to centroids of the intrinsic subtype and can calculate a risk-of-recurrence (ror) score that incorporates a proliferation score and gross tumour size into the algorithm.

The research version that preceded the Prosigna genomic assay is the PAM50 and its associated ror score. The PAM50 ror score has been assessed for independent prognostic impact beyond the traditional clinical and pathologic risk factors for outcome in postmenopausal early-stage hormone receptor–positive bca treated with adjuvant hormonal therapy alone (no adjuvant chemotherapy).^6,15 The PAM50 ror score has been investigated for predicting both early and late distant recurrences. In the ABCSG-8 trial, which compared 5 years of tamoxifen with 2 years of tamoxifen followed by 3 years of anastrozole, tumour samples from 1478 of the 3901 patients in the study were available and suitable for PAM50 assay. In that correlative study, 29% of the cohort had node-positive disease, and 5% were HER2-positive. Using regression modelling, the authors found that the ror score added significant prognostic information beyond the clinical parameters.
The probability of 10-year distant relapse-free survival was 96.7% (95% CI: 94.6% to 98%) in the low-risk cohort, 91.3% (95% CI: 88.1% to 93.8%) in the intermediate nodal group, and 79.9% (95% CI: 75.7% to 83.4%) in the high-risk group. By subtyping categorization alone, the 10-year risk for distant relapse was lower for luminal A relative to luminal B cancer (hazard ratio: 2.85), with 10-year distant relapse-free survival rates of 93.9% (95% CI: 92% to 95.3%) and 82.2% (95% CI: 77.8% to 85.8%) respectively.

The prognostic value of the PAM50 nod score to predict late recurrences (defined as more than 5 years after diagnosis and disease-free, and after 5 years of endocrine treatment) was assessed in a combined analysis of the ABCSG-8 trial and the TRANSATAC trial. The investigators were able to obtain long-term clinical outcomes data and tissue samples from 2137 postmenopausal women enrolled on the two trials (none of whom had received adjuvant chemotherapy). They found that the Clinical Treatment Score, which incorporates nodal status, tumour size and grade, age, and treatment, provided the strongest prognostic information. However, the nod score was additionally prognostic, particularly in the node-negative cohort. The risk of a distant recurrence between years 5 and 10 was 2.4% (95% CI: 1.6% to 3.5%) in the nod low-risk group, 8.3% (95% CI: 6.1% to 11.2%) in the nod intermediate-risk group, and 16.6% (95% CI: 13.1% to 20.9%) in the nod high-risk group. It is important to emphasize that the information provided is prognostic and not necessarily predictive. That is, the study results do not imply that, compared with a nod low-risk cohort, a nod high-risk cohort would differentially benefit to a greater relative degree from extended hormonal therapy. Rather, if Prosigina were to be used to provide prognostic information, it can be interpreted as suggesting the risk of late recurrence and the potential absolute reduction to be obtained with extended hormonal therapy. It would appear that, in patients having tumours with a low nod score, the absolute risk of a distant event during years 5–10 would not warrant consideration of extended hormonal therapy (with either tamoxifen or an aromatase inhibitor).

Lastly, a correlative study led by the Danish Breast Cancer Group in which postmenopausal women in Denmark who had “high risk” disease (defined as positive in 1–3 nodes, or node-negative with either a tumour size > 2 cm or a tumour grade of 2–3) and who were treated during 1999–2003 according to national guidelines (hormonal therapy alone) undertook a retrospective tumour analysis using the Prosigina assay. Of the 2749 blocks collected, 1480 came from patients with node-negative disease. Again, the nod score was prognostic in the entire study cohort, but notably, either a low nod score or a subtype designation of luminal A suggested a relatively good prognosis in the node-positive cohort, with 10-year distant relapse rates of 4.8% (95% CI: 3.1% to 6.9%) and 8.7% (95% CI: 6.7% to 10.9%) respectively.

**MammaPrint**

MammaPrint is a 70-gene expression signature that was initially developed and applied in fresh-frozen tissue to reveal either a good or a poor prognostic signature in early-stage breast cancer. The assay was tested on a series of samples from 295 consecutive patients in the Netherlands Cancer Institute fresh-frozen tumour bank. Patients were under the age of 52 years, with 51% having N0 disease and the remainder (n = 144) having node-positive disease. Just a small number of patients in the N0 cohort (10 of 151) received adjuvant systemic therapy; most patients in the node-positive cohort (120 of 144) received such therapy (predominantly chemotherapy, with or without hormonal therapy). At 10 years, the distant disease-free survival was 50.6% ± 4.5% in the group with a poor-prognosis signature and 85.2% ± 4.3% in the group with a good-prognosis signature. A subsequent validation study confirmed the good- and poor-prognosis significance of this 70-gene signature.

The early work on MammaPrint subsequently led to a prospective phase III trial in 6693 women with early-stage breast cancer (MINDACT, NCT00433589 at http://ClinicalTrials.gov) that compared clinical risk (modified version of Adjuvant! Online) and genomic risk (MammaPrint) with respect to prognosis scoring in cases of concordant high- or low-risk results and prediction of adjuvant chemotherapy benefit in discordant high- or low-risk results. The study included patients with node-positive (21% of the study population) and node-negative disease, an ER-negative population (12% of the study population) and a HER2-positive population (10% of the study population). Median follow-up was 5 years.

The primary analysis in MINDACT assessed for a non-inferiority bound of 92% in 5-year distant metastasis-free survival for the patients who were determined to be clinically high-risk, but genomically low-risk, and who were treated without adjuvant chemotherapy. That specific cohort included 1550 patients, of whom 98% were ER-positive, 8% were HER2-positive, and 48% were node-positive. The 5-year distant metastasis-free survival without adjuvant chemotherapy was 94.7% (95% CI: 92.5% to 96.2%). Thus, the study met its primary statistical endpoint. However, surprisingly, in the discordant cohort (clinically low risk, but genomically high risk), the randomization to chemotherapy or no chemotherapy resulted in a hazard ratio of 1.17 (95% CI: 0.59 to 2.28) with a 5-year distant metastasis-free survival of 95% in the no-chemotherapy arm (95% CI: 91.8% to 97%) and 95.8% in the chemotherapy arm (95% CI: 92.9% to 97.6%).

That lack of a positive interaction test for the 70-gene signature fails to support any predictive significance for the assay in respect to benefit with adjuvant chemotherapy. The study does support a prognostic impact for the 70-gene signature in addition to standard clinicopathologic risk parameters for identifying a population that might be able to be spared adjuvant chemotherapy. However, it does not appear to support the ability of a high-risk 70-gene signature to predict a greater differential benefit with chemotherapy.

**EndoPredict**

EndoPredict is another commercialized multigene assay that can be performed using FFPE tissue. It quantifies 8 genes of interest by quantitative reverse-transcriptase polymerase chain reaction. During development of the assay, investigators determined that the combination of EndoPredict with two clinical factors (nodal status and tumour size)—the “EPclin score”—improves the prognostic discrimination of the test over EndoPredict alone. The EPclin score classifies tumours into two risk groups: low-risk for distant recurrence (<3.3) and high-risk for distant recurrence (>3.3).
EndoPredict has been retrospectively assessed in two randomized phase III trials (ABCSG-6 and ABCSG-8) involving 1702 postmenopausal patients with ER-positive, HER2-negative early-stage BCa treated with adjuvant hormonal therapy alone (tamoxifen alone for 5 years, or tamoxifen for 2 years followed by anastrozole for 3 years)3. Investigators found that the EPclin score added prognostic information beyond that available with the standard clinicopathologic parameters, including Ki-67 status, identifying a cohort with a low risk of distant relapse at 10 years. Furthermore, in comparing various consensus guidelines (U.S. National Comprehensive Cancer Network, German S3, and St. Gallen) with EPclin, the EPclin reclassified between 58% and 61% of women out of the high- or intermediate-risk groups (according to clinical guidelines) to low-risk (according to EPclin). The reclassification of the women was associated with a 5% rate of distant relapse at 10 years when treated with 5 years of hormonal therapy alone. Thus, it appears that EPclin is another genomic assay–based score that can identify a low-risk cohort of women with ER-positive BCa who will have a relatively good prognosis when not treated with adjuvant chemotherapy.

Breast Cancer Index

The Breast Cancer Index (BCI) is an quantitative reverse-transcriptase polymerase chain reaction assay that measures 7 genes (in addition to housekeeping genes) primarily related to estrogen-related signalling (HOXB13:IL17BR ratio) and proliferation (molecular grade index)19. As with the assays already discussed, the BCI has been demonstrated to have independent prognostic capacity to identify a cohort of node-negative patients with a relatively good prognosis out to 5 and 10 years of follow-up when treated with adjuvant endocrine therapy alone20.

In an attempt to predict for a differential benefit of extended adjuvant hormonal therapy, the BCI assay has also been taken further in a nested case-control study based on FFPE material from the NCIC’s MA.17 trial21. The randomized placebo-controlled MA.17 trial demonstrated that, compared with placebo, 5 years of letrozole in postmenopausal women who had completed 4.5–6 years of adjuvant tamoxifen was associated with improved disease-free survival21. Investigators found that patients with a high BCI score (HOXB13:IL17BR ratio) experienced a 5-year absolute disease-free survival benefit of 16.5% from extended endocrine therapy with letrozole (p = 0.007); patients with a low BCI score appeared to experience no significant benefit from extended endocrine therapy with letrozole (p = 0.35)10. The interaction test to assess for the predictive significance of the BCI with respect to benefit from extended hormonal therapy was significant (p = 0.03).

Genomic Grade Index

The Genomic Grade Index (GGI) is a 97-gene microarray signature developed with the goal of segregating an intermediate histologic grade invasive BCa into either a prognostic low-grade or high-grade natural history category in both tamoxifen-treated and -untreated patients22. Most of the work with the GGI has aimed at correlating it with the pathologic response to neoadjuvant chemotherapy. In a correlative study involving 229 patients treated with neoadjuvant anthracyclines and taxanes, a high GGI was associated with a greater chance of achieving a pathologic complete response in both hormone receptor–positive and –negative tumors23. A high GGI was also associated with worse distant relapse-free survival in the hormone receptor–positive cohort.

SUMMARY

Clearly, the biology of BCa is not limited to anatomic risk factors, particularly with respect to prognosis with adjuvant endocrine therapy or prediction of benefit with adjuvant chemotherapy. The genomic assays outlined in the present review all demonstrate an ability to provide independent prognostic value beyond the standard clinical and pathologic risk factors, and all are now adopted in expert consensus guidelines24,25.

Despite differences in genes sets and platforms, each assay can identify a cohort of patients who, when treated with 5 years of endocrine therapy alone, have a relatively low risk of distant relapse, such that any marginal benefit of adjuvant chemotherapy is unlikely to outweigh its long-term toxicities. However, not all assays have demonstrated the ability to differentiate the magnitude of the potential benefit with adjuvant chemotherapy. This latter aspect is arguably of greater added value for clinical scenarios in which adjuvant chemotherapy is considered to be a therapeutic option in early-stage hormone receptor–positive BCa—which is likely the reason that the clinical practice guideline from the American Society of Clinical Oncology strongly recommends the Oncotype dx and the PAM50 nor score be used to guide decisions about adjuvant systemic therapy in ER-positive, HER2-negative, node-negative BCa25. Prospective validation of Oncotype dx for the prediction of benefit (or lack thereof) for adjuvant chemotherapy in ER-positive, HER2-negative, node-negative BCa with a risk of 11–25 from the TAILORx study is awaited. Also awaited are data from the RXPONDER trial (NCT01272037 at http://ClinicalTrials.gov) to define the role of Oncotype dx in ER-positive, HER2-negative BCa with 1–3 involved nodes and a risk of 25 or less. New therapeutic agents and strategies have resulted in improved BCa survival. Moving forward, the increasing use of genomic assays will permit health care providers to individualize therapy (type of therapy or de-escalation), resulting in optimization of clinic benefit with minimization of short- and long-term toxicities from systemic therapies.

CONFLICT OF INTEREST DISCLOSURES

I have read and understood Current Oncology’s policy on disclosing conflicts of interest, and I declare the following interests: I have received fees as an advisory board member for Genomic Health and NanoString. My institution receives funding from Genomic Health for a trial in which I am a co-investigator.

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