Genome-Based Risk Prediction for Early Stage Breast Cancer

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ABSTRACT

Tests to better characterize tumor genomic architecture are quickly becoming a standard of care in oncology. For breast cancer, the use of gene expression assays for early stage disease is already common practice. These tests have found a place in risk stratifying the heterogeneous group of stage I–II breast cancers for recurrence, for predicting chemotherapy response, and for predicting breast cancer-related mortality. In the last 5 years, more assays have become available to the practicing

oncologist. Given the rapidity with which this field has evolved, it is prudent to review the tests, their indications, and the studies from which they have been validated. We present a comprehensive review of the available gene expression assays for early stage breast cancer. We review data for several individual tests and comparative studies looking at risk prediction and cost-effectiveness. *The Oncologist* 2014; 19:1019–1027

Implications for Practice: Gene expression assays are an important component in the management of early stage breast cancer. Understanding their utility as well as their drawbacks is critical for the practicing oncologist to make informed decisions regarding care.

INTRODUCTION

Breast cancer is one of the leading causes of cancer-related morbidity worldwide [1]. In the U.S., 235,030 new cases are expected to be diagnosed in 2014 [2]. Of these, approximately 164,000 cases (70%) will be classified as early stage (stage I–II) breast cancer, for which the potential for cure is excellent. Although surgery is the major curative modality, adjuvant chemotherapy plays an important role in increasing cure rates for a selected group of patients. Approximately 15%–30% of patients with stage I breast cancer and up to 40% of stage II node-negative patients will have a recurrence with local therapy alone. The disparity in outcomes within groups of similar histopathologic characteristics speaks to the incredible heterogeneity of this disease [3, 4]. How to better define and identify these higher risk individuals has been an area of intense investigation for more than a decade [5].

This clinical conundrum has resulted in a scientific race of sorts to more precisely prognosticate risk of recurrence (ROR) in early stage breast cancer. The investigation has focused mainly on patients with hormone receptor-positive (HR+) HER2-negative (HER2–), and lymph node-negative (LN–) disease, encompassing the majority of patients with early stage breast cancer. HER2-positive tumors are considered a separate group that benefit from adjuvant HER2-based chemotherapy [6–8]. Axillary lymph node involvement is associated with poor outcome, and those patients generally

receive adjuvant chemotherapy. However, efforts are ongoing to identify patients with one to three positive lymph nodes for whom chemotherapy is not indicated. This is particularly important because although the benefits of chemotherapy may be great for a subset of patients, chemotherapy may also confer significant, potentially life-long toxicities for others. These include peripheral neuropathy; premature ovarian failure; and, rarely, cardiac dysfunction [9, 10]. Short-term toxicities are also substantial. Risk for infection, alopecia, and fatigue, in addition to the emotional toll that chemotherapy can often take on patients, are important side effects, and all may substantially affect quality of life. These secondary effects are common to most standard adjuvant chemotherapy regimens for breast cancer [11].

Focus has been on the larger group of stage I–II, node-negative, HR+, HER2– breast cancer patients for two reasons: to better communicate with patients about true individual risk of recurrence and to better identify high-risk patients for whom adjuvant chemotherapy may be more recommended and low-risk patients for whom chemotherapy may offer little benefit.

In order to have a more concrete understanding of the risks and benefits of adjuvant chemotherapy, several gene expression assays have been developed to better stratify this group of diverse patients. The assays evaluate varying numbers of genes

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in the breast tumor, to quantify their expression levels, and output a score that correlates with risk of recurrence. These tests, which are commercially available and in some cases are covered by insurance in the U.S., are being used in clinical practice to assist with prognostication and often to aid decision making regarding adjuvant chemotherapy.

Since the last time *The Oncologist* reviewed gene expression assays for breast cancer, in 2008, more tests have become commercially available, and multiple validation studies have been published [12]. In addition, one such test was cleared by the U.S. Food and Drug Administration (FDA) in September 2013, and others are actively seeking approval by regulatory agencies. Given the large number of options now available to practicing oncologists, it is pertinent to update this review with a comprehensive analysis of the available breast cancer gene expression tests. We aim to provide a clearer understanding of these assays so that providers may make more informed decisions in the clinic, avoid unnecessary use of cytotoxic chemotherapy, and reduce the burden on the health care system.

MATERIALS AND METHODS

A comprehensive literature search of all relevant clinical trials, original research articles, review articles, and editorials in the PubMed database concerning gene expression assays in breast cancer was performed. Original research publications pertaining to discovery-phase and validation studies for each of the specified gene expression assays were reviewed. The search terms “gene expression profiling,” “gene expression assay,” “gene expression,” “Oncotype DX,” “MammaPrint,” “PAM50,” “Breast Cancer Index,” and “breast cancer” were used. Individual biotechnology company websites were also reviewed for additional information regarding tissue requirements, technology used, and availability of tests. Assays based on gene expression profiling were included. Those based on immunohistochemistry (IHC) were excluded. Tests that are clinically available in the U.S. were included (Table 1). Those available only in Europe, such as MapQuant Dx and EndoPredict, were excluded.

GENE EXPRESSION ASSAYS

Oncotype DX

This 21-gene assay developed by Genomic Health (Redwood City, CA, <http://www.genomichealth.com>) is the most frequently used test in clinical practice in the U.S. [13]. Based on quantitative reverse transcription polymerase chain reaction (PCR) expression levels of 5 reference genes and 16 selected genes related mostly to the estrogen receptor (ER), HER2, proliferation, and invasion, the assay determines a recurrence score (RS) that assigns patients into a low-, intermediate-, or high-risk category. The original training set with which the assay was designed used 447 patient specimens from three breast cancer clinical trials (a variety of patients, some with ER- and LN+ disease) but was most heavily weighted toward the tamoxifen-only arm of the National Surgical Breast and Bowel Project-B20 (NSABP-B20) cohort [14]. The training set included 250 candidate genes that were eventually paired down to 21 based on a best-fit model. The assay was validated in a study published by Paik et al. in the *New England Journal of Medicine* in 2004 [14]. The validation cohort included 668 ER+, LN- patients from the NSABP-B14 study who were all treated with

tamoxifen. The study showed a significant difference in distant recurrence at 10 years among the three groups, as defined by the RS, and the score provided predictive power independent of tumor size and age. The distant recurrence rate at 10 years was 6.8% for the low-risk group, 14.3% for the intermediate-risk group, and 30.5% for the high-risk group. The RS was also shown to correlate independently with overall survival (OS).

The test has since been validated in multiple other studies, especially for patients treated with endocrine therapy. Among these is another study by Paik et al., from 2006, demonstrating the use of the Oncotype DX assay not only for prediction of recurrence but also for prediction of chemotherapy benefit [15]. Again, using the NSABP-B20 cohort, in this study Paik et al. evaluated 227 patients randomized to treatment with tamoxifen alone versus 424 patients treated with tamoxifen plus chemotherapy (cyclophosphamide, methotrexate, and 5-fluorouracil [5-FU] or methotrexate and 5-FU). The RS significantly correlated with benefit from chemotherapy as determined by 10-year disease-free survival (DFS) compared between each RS group in the tamoxifen-only and tamoxifen-plus-chemotherapy arms. It was estimated that freedom from distant recurrence was increased from 60% to 88% when chemotherapy was added to tamoxifen in the high-risk RS group. The benefit of chemotherapy in the intermediate-risk group was less clear. However, it is important to note that, similar to MammaPrint, this study was not an independent validation because there was duplication of samples from the training set. Only one of the original validation studies showed no prognostic value in patients with node-negative breast cancer who received no adjuvant systemic therapy [16]. However, the study included patients with ER+ and ER- breast cancer, and the test was originally designed to assess ER+ tumors. Nevertheless, given the aforementioned studies and similar data from other validations, the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network guidelines have recommended the use of Oncotype DX to risk-stratify patients with ER+, HER2-, LN-/N1mi (axillary metastases <2 mm) early stage breast cancer [17–20].

PAM50

Prosigna (NanoString Technologies Inc., Seattle, WA, <http://www.nanostring.com>) is the gene expression profile assay most recently cleared by the FDA, gaining approval in September 2013. The technology, unlike other assays, can be performed in any CLIA-certified laboratory with the use of NanoString's NanoCounter Analysis System. Briefly, gene expression is measured in RNA extracted from formalin-fixed paraffin-embedded tissue using a novel, digital, color-coded, bar code technology that allows measurement of multiple transcripts with high sensitivity (less than one copy per cell). A panel of 50 classifier genes and 5 normal genes was selected to classify breast cancers into an intrinsic subtype (luminal A, luminal B, HER2-enriched, and basal-like). Using the intrinsic subtype defined by the 50-gene signature, along with standard prognostic parameters, multiple studies have shown PAM50's improved ability to prognosticate risk of recurrence. The training set for PAM50 included 29 normal breast samples and 189 tumors with approximately 50% LN+ disease and mostly high-grade tumors [21]. The training set evaluated 1,906 genes, and this number was ultimately reduced to 50 genes

Table 1. Breast cancer gene expression tests clinically available in the U.S.

Test	Company	Number of genes	Tissue	Technology	Measure	FDA-cleared
Prosigna	Nanostring	50 + 22 ctl ^a	FFPE	Digital bar-coded mRNA analysis	ROR: Low (<10), intermediate (10–20), high (>20%) risk	Yes
Oncotype DX	Genomic Health	16 + 5 ctl	FFPE	qRT-PCR	RS: low (<18), intermediate (18–31), high (>31) risk	No
MammaPrint	Agendia	70	Fresh frozen, FFPE	Microarray	Good risk and poor risk	Yes
Breast Cancer Index	bioTheranostics	5 + 2 gene ratio	FFPE	qRT-PCR	Low, intermediate, and high risk	No

^aProsigna measures the expression levels of 50 genes used in the PAM50 classification algorithm, 8 housekeeping genes used for signal normalization, 6 positive controls, and 8 negative controls.

Abbreviations: ctl, control/housekeeping genes; FDA, U.S. Food and Drug Administration; FFPE, formalin-fixed paraffin-embedded; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; ROR, risk of recurrence; RS, recurrence score.

of interest with 8 controls. This 50-gene classifier was tested on 761 samples from patients who had not received adjuvant systemic therapy and 133 who had received neoadjuvant chemotherapy (paclitaxel followed by 5-FU, doxorubicin and cyclophosphamide). In the first group of no adjuvant therapy, the majority of the samples were node-negative (710 of 761). The 50-gene classifier showed a clear difference in recurrence-free survival (RFS) in the 4 identified intrinsic subtype groups. These intrinsic subtypes do not correspond completely with ER and HER2 status based on IHC [22], suggesting that the subtype is not simply a recapitulation of findings based on routine histologic examination. The statistically significant difference in RFS remained after groups were stratified by ER status. The identified subtypes, however, were less successful at predicting outcomes for HER2+ tumors. Prediction for risk of recurrence was significantly improved when the subtype classification was added to known prognostic clinical variables. The combination of these parameters was coined “ROR-C,” risk of recurrence plus clinical variables. In addition, in the group of patients who received neoadjuvant therapy, risk of recurrence based on intrinsic subtypes was able to predict pathologic complete response to chemotherapy with 94% sensitivity and 97% negative predictive value [21]. Nielsen et al. validated these findings in an independent cohort of 786 patients with both LN+ and LN– disease who were treated with tamoxifen only [23]. The majority of the tumors were ER+. The intrinsic subtypes and the ROR scores identified by PAM50 were strongly prognostic for RFS and disease-specific survival. An even larger validation study was published more recently, in January 2014, looking at 1,478 patients from the Austrian Breast and Colorectal Study Group 8 (ABCSG-8) trial population [24]. This cohort included postmenopausal ER+ early stage breast cancer patients who received adjuvant hormonal therapy (tamoxifen alone or tamoxifen followed by aromatase inhibitor) without adjuvant chemotherapy. Again, the PAM50 ROR significantly predicted RFS. In all subgroups, except for the HER2+ group, the ROR score and ROR risk groups added prognostic information to the routinely used clinicopathologic parameters. Currently, PAM50 is approved for use in postmenopausal women with HR+ tumors with or without lymph node involvement.

MammaPrint

MammaPrint (Agendia, Amsterdam, The Netherlands, <http://www.agendia.com>) was the first gene expression array for

breast cancer prognosis to be cleared by the FDA, back in February 2007. MammaPrint is composed of a 70-gene microarray. The original training set evaluated 25,000 genes in 78 LN– sporadic early breast cancer tumors that were <5 cm in diameter and present in patients <55 years old [25]. The genes were eventually narrowed to a 70-gene classifier that showed a difference in expression patterns between good-prognosis and poor-prognosis groups, as defined by the authors. However, the groups were preselected based on their clinical outcomes (one group with distant metastases at 5 years, the other disease free at 5 years). A subsequent validation study assessed 295 patients with pT1 or pT2 tumors with pN0 or pN+, with a median follow-up of 7.8 years [26]. Sixty-one of the 295 patients were part of the training set; therefore, this was not a completely independent validation set, although a separate analysis was also completed leaving out the repeated tumors. The analysis showed a significant difference between OS and metastasis-free survival in the good- and poor-prognosis groups. Overall survival across all patients, comparing the good-prognosis and poor-prognosis groups, was 94% versus 54%, respectively, at 10 years. When evaluating the LN+ patients alone, the MammaPrint signature continued to be a significant prognostic marker. This 70-gene signature has since been validated in other studies, including patients with up to 9 positive lymph nodes [27–30]. In the past, limitations of the assay included the requirement for fresh frozen tissue and high-quality cDNA; however, since 2012, Agendia has made the test available for formalin-fixed paraffin-embedded tissue [31].

Breast Cancer Index

The Breast Cancer Index (BCI) (bioTheranostics, San Diego, CA, <http://www.biotheranostics.com>) incorporates two previously established gene expression assays into one test. It evaluates the ratio of the expression of two genes, *HOXB13* and *IL17BR*, based on qualitative PCR in combination with the Molecular Grade Index. The Molecular Grade Index is a five-gene expression assay looking at genes related to histologic grade and tumor progression. In an initial analysis, Ma et al. demonstrated that in 60 postmenopausal ER+ patients treated with 5 years of tamoxifen, the ratio of *HOXB13* to *IL17BR* was an independent prognostic factor, with higher ratios correlating with poorer outcomes [32]. The study also showed that the ratio was able to predict for response to

tamoxifen therapy. Later, it was shown that the combination of this two-gene ratio with the Molecular Grade Index provided better predictive value than either alone. This finding was validated in a cohort of 588 postmenopausal early stage ER+ breast cancer patients from the Stockholm trial of adjuvant tamoxifen versus placebo [33]. This study was used to develop a continuous risk predictor, the BCI, using a scale of 0 to 10. The BCI was prognostic in an independent population of node-negative patients who did not receive adjuvant therapy, with the aim of risk stratifying for breast cancer death [34]. The predictive role of the BCI in patients receiving extended adjuvant endocrine therapy was tested on tissue from patients who participated in the MA.17 trial. In this study, high BCI was associated with a decrease in late recurrence in patients receiving extended letrozole therapy [35]. The BCI test may be considered to assess the probability of distant recurrence in patients diagnosed with ER+ node-negative breast cancer and to predict the likelihood of benefit from extended (>5-year) endocrine therapy in patients who are recurrence free after an initial 5 years of adjuvant endocrine therapy.

COMPARATIVE STUDIES

Given the wealth of data supporting each individual test, how does one choose which to use in clinical practice? Only a handful of studies have compared the assays to one another. One such study evaluated 1,017 ER+ early stage breast cancer patients from the Arimidex, Tamoxifen Alone or in Combination (ATAC) trial [36]. In this study, Oncotype DX was compared with PAM50 and IHC4 (an integrated score of the immunohistochemical markers Ki-67, ER, PR, and HER2). The primary endpoint was distant recurrence after endocrine therapy. The authors concluded that the PAM50 ROR, compared with the Oncotype DX RS, provided more prognostic information regarding risk of recurrence and better differentiated the intermediate- and high-risk groups.

In the same year, the *Lancet* published an article comparing the BCI with Oncotype DX and IHC4, using the translational arm of the ATAC (TransATAC) population. The study looked at both early (within the first 5 years) and late (after 5–10 years) distant recurrence. The analysis included 665 patients. The authors suggested that the BCI, when used as a linear model, was more predictive than Oncotype DX (hazard ratio: 1.69) and that only the BCI was able to predict for late distant recurrence [37].

An interesting study performed years earlier evaluated the concordance between various gene expression arrays. In 2006, Fan et al. compared 5 gene sets, among which were the MammaPrint 70-gene set, the Oncotype DX RS 21-gene set, the intrinsic subtypes as defined by Perou et al., and the ratio of HOXB13 to IL17BR, which makes up part of the BCI [38]. The study was based on an independent set of 295 breast cancer cases for which gene expression microarrays were performed. The investigators performed multiple microarrays on each sample using the various gene sets mentioned above. Although the population used was a heterogeneous one, including ER[−] cancers and a variety of adjuvant treatments, Fan and collaborators showed that for both the composite group and the ER+ patients alone, the RFS and OS as predicted by the tests were similar in four of five of the gene expression arrays. The two-gene ratio failed to distinguish outcomes between the two high-risk and low-risk groups it had

identified. The study also revealed that the 70-gene profile and the 21-gene RS classify similar groups of patients into risk categories (77% concordance for ER+ patients and 81% concordance overall). When looking at the intrinsic subtypes, the 21-gene RS and 70-gene profile classify all of the basal-like subtypes into the high-risk category and have similar trends for the other subtypes (Fig. 1). This study suggests that although the actual genes tested are not the same, similar biological characteristics are being tested in the gene expression assays and generate similar outcomes in prognostication.

COST-EFFECTIVENESS ANALYSES

With recent cutbacks in health care expenditure in the U.S., practitioners are increasingly called on to make cost-effective decisions. This can often be difficult, given the hefty price tag of genomic testing and insurance restrictions. Fortunately, several cost-effectiveness analyses have been performed to assess the available gene expression assays. Adjuvant! Online, a publicly available online risk predictor, is often used as a standard with which to compare the cost-effectiveness of gene expression assays because it incorporates patient and tumor characteristics (e.g., age, LN status, tumor size) into its prognostication model and is free.

In the U.S., Oncotype DX and MammaPrint have both been shown independently to be cost-effective tests in guiding decisions regarding adjuvant therapy [39, 40]. More important, a cost-effectiveness analysis directly comparing Oncotype DX with MammaPrint was performed in 2012. The analysis compared the cost and quality-adjusted life years (QALYs) of each test. Based on a hypothetical cohort of ER+, LN[−] early stage breast cancer patients, the study concluded that MammaPrint is the most cost-effective test based on a willingness-to-pay threshold of \$50,000 per QALY [13].

In the U.K., another cost-effectiveness analysis looked at nine different gene expression profiles and IHC-based tests including Oncotype DX, MammaPrint, the BCI, and PAM50 [41]. The study reviewed prior analyses and developed a model to evaluate the cost-effectiveness of adjuvant therapy guided by the tests as compared with routine care guided by standard clinicopathologic criteria. Unfortunately, the authors found that data were insufficient to fully compare each of the tests. They suggested that IHC4 had the most potential to be cost-effective; however, evidence for its clinical utility equal to or beyond the genomic-based tests was lacking.

UNRESOLVED QUESTIONS AND ANTICIPATED STUDIES USING GENE EXPRESSION ASSAYS

Multigene assays are currently being used in large part to estimate risk of recurrence in patients with ER+/HER2[−] tumors; however, the definition of ER positivity is evolving. Traditionally, tumors were considered ER+ and/or PR+ if at least 10% of the cells exhibited protein expression using IHC. In 2010, a joint committee from ASCO and the American College of Pathology recommended a 1% cutoff for ER and PR positivity [42]. This recommendation was based on data from a SWOG study conducted decades ago [43], and, consequently, the prognostic and predictive value of ER and PR between 1%–9% remains poorly defined [44].

Given that these assays are already being used in clinical practice, several clinical trials have incorporated them into

Figure 1. Number of patients per intrinsic subtype and their risk categories as determined by the 70-gene profile and RS (21-gene profile) derived from microarray data. Within each intrinsic subtype, risk categories per patient are similar between assays, although complete concordance is seen only for the basal-like group. Adapted from data in Table 2 of Fan et al. [36].

Abbreviation: RS, recurrence score.

their designs to answer further questions about their utility (Fig. 2). The TAILORx trial, which has completed accrual and is in the analysis phase, will look at the utility of chemotherapy specifically in patients with an intermediate RS (Oncotype DX) [45]. The results of this trial are highly anticipated because there is no standard of care for these intermediate-risk patients, and decisions are largely dependent on shared decision making between the provider and the patient.

A similar SWOG trial is ongoing for patients with ER+, HER2- breast cancer and one to three positive nodes. The RxPONDER trial randomly assigns patients with an RS ≤ 25 to endocrine therapy alone or to chemotherapy plus endocrine therapy [46]. This trial is currently accruing patients, with the goal of randomizing 4,000 patients, and aims to answer the same question as the TAILORx trial with regard to the benefit of chemotherapy in patients with low to intermediate RS but specifically for node-positive patients.

In line with aims at further personalizing oncologic care, more precise prognostication is needed to meet the individual needs of patients. Gene expression assays have the potential to fill the gap where clinicopathologic criteria fall short.

The MINDACT trial is another large randomized clinical trial that has incorporated MammaPrint into the design. The trial evaluates the clinical utility of MammaPrint (genomic factors [G]) in combination with clinicopathologic risk factors assessed by Adjuvant! Online (clinical factors [C]). The study incorporates early stage breast cancer cases that are LN- or that have one to three positive lymph nodes. The design is

complex and not only looks at MammaPrint utility but also compares adjuvant chemotherapy and adjuvant endocrine regimens. In order to evaluate MammaPrint utility, patients who have discordant G and C results are randomized to adjuvant chemotherapy or no chemotherapy. Recently, the pilot phase, which comprised 800 patients, was published. In this first initial cohort of patients, 27% had discordant G and C risks and approximately half received chemotherapy. The pilot phase demonstrated that the study is feasible, and the outcomes are expected to be quite informative [47].

Last, the OPTIMA trial is a randomized phase III study that will compare outcomes of test-directed treatment with a control arm of chemotherapy plus endocrine therapy for pN1-2 or pT3N0 ER+, HER2- breast cancers. In addition to clinical outcomes, the study will evaluate cost-effectiveness and health resource utilization related to multigene assays for early stage breast cancer in the U.K. [48].

DISCUSSION

Although the vast majority of stage I-II HR+, HER2- patients will never develop recurrent disease, the fear of recurrence often drives patients and providers to choose adjuvant chemotherapy. The majority of patients receive no benefit from this treatment; however, this practice continues because current prognostic markers fail to identify which patients within this group are at greatest risk. In line with aims at further personalizing oncologic care, more precise prognostication is needed to meet the individual needs of patients. Gene expression assays have the potential to fill the gap where clinicopathologic criteria fall short. Figure 3 shows a proposed algorithm for clinical decision making using available multi-gene expression assays.

Figure 2. Overview of clinical trials that have incorporated currently available assays into their designs to answer further questions about the assays' utility.

Abbreviations: CT, chemotherapy; HR, hormone receptor; IC, informed consent; PIS, patient information sheet; R-C, chemotherapy randomization of anthracycline-based CT (FEC → D for N+ docetaxel/capecitabine); R-E, endocrine treatment randomization, letrozole vs. tamoxifen followed by letrozole; R-T, treatment decision randomization based on genomic vs. clinical prognosis; RS, recurrence score.

Figure 3. Suggested schema for clinical decision making.

We have reviewed the four main gene expression assays used in the U.S. to evaluate risk of recurrence in early stage breast cancer patients with HR+, LN– disease. The assays reviewed in this article each have their own individual advantages. Oncotype DX has perhaps the strongest clinical evidence, with 13 retrospective studies, totaling >4,000 patients, suggesting it is clinically useful. The assay has been shown to be prognostic, and, to some degree, it may predict chemotherapy response.

PAM50, the newcomer to the group, has been validated in large cohorts (>1,000 patients). The bulk of the data support its prognostic value in patients with early stage, ER+/HER2– breast cancer. Emerging, albeit limited, data showed that PAM50 may also be predictive of response to neoadjuvant chemotherapy, although more studies are needed to validate its predictive role in this setting [49]. One of the potential advantages of the PAM50 assay is that it can be performed and reported by hospital and commercial laboratories. Although reproducibility and technical expertise within a laboratory certified under the Clinical Laboratory Improvement Amendments of 1988 is necessary, the ability to offer testing within one's own institution has multiple benefits including personalized overview of testing, cost control, likely faster turn-around time, and an opportunity for institutional data collection. It should be noted that the FDA cleared only the Prosigna ROR score for clinical use; therefore, reports from commercial laboratories may not include the intrinsic subtype information.

This skepticism regarding the clinical utility of molecular subtypes was reiterated in a publication from the *Annals of Oncology* that evaluated the medical usefulness of the intrinsic subtypes as defined by PAM50 compared with IHC-classified breast cancer subtypes (HER2 and HR status) [50]. An expert panel, the IMPAKT task force, composed of pathologists, clinicians, biostatisticians, and scientists, reviewed literature to determine evidence for the use of PAM50 molecular

subtypes in clinical practice. After a thorough review of the evidence, the group determined that data were insufficient to support the clinical validity and utility of PAM50 molecular subtypes. They recommended against using the intrinsic subtypes to guide treatment decisions, in part because there may be some discrepancy between intrinsic subtypes defined by PAM50 compared with IHC-defined classes, which were considered to be more strongly validated tests. These recommendations fall in line with the FDA recommendations for use of this assay. However, the IMPAKT task force did not address the utility of the PAM50 ROR score, which should be considered separately and which we feel has been well established based on the available data.

Looking at all four of the assays reviewed in this paper, we return to the study by Fan et al., which was the only one to evaluate all of these assays together in a single investigation [39]. Fan et al. used the gene sets from each assay (MammaPrint, Oncotype DX, PAM50, and the two-gene ratio from the BCI) to compare predicted outcomes for an independent set of breast cancers. It is important to note that the gene set for the PAM50 ROR was not compared with the other assays in this study but rather the intrinsic subtypes from PAM50 were used. The study concluded that the gene sets as a whole identified similar groups of high-risk and low-risk cancers, although 100% concordance was not seen. The largest discrepancy was within the luminal A group, in which some cases identified as low risk by the MammaPrint, or 70-gene, assay were classified as intermediate risk by the Oncotype DX, or 21-gene, assay. The purpose of this study was not to determine which assay was most predictive of outcomes but rather to evaluate the inherent concordance between gene sets. Although interesting in terms of biological principles, underscoring the concept that the assays are ultimately measuring the same tumor properties, the study does not distinguish one assay from another in terms of usefulness for clinical practice.

Perhaps the most useful study for directing clinical decision making, in our opinion, was the head-to-head comparison of PAM50 and Oncotype DX [36]. In this investigation, PAM50 provided more prognostic information than Oncotype DX with regard to likelihood ratios and distant recurrence rates over a median of 10 years of follow-up. Comparison indices revealed that the PAM50 ROR outperformed the Oncotype DX RS for every tumor subtype. In addition, hazard ratios for the low- and high-risk groups categorized by ROR were improved compared with the low- and high-risk groups identified by the RS. Although this is only a single study, it provides high-quality data with which to compare the two tests.

That being said, a major drawback of all of the gene expression assays reviewed is that HER2+ and LN+ patients were included in the test cohorts. By including these higher risk individuals, the recurrence scores may have less inherent clinical utility because they are currently only being applied to HR+, HER2–, LN– disease. Including HER2+ and LN+ high-risk patients in the test cohort may bias the analysis and compromise the ability to determine whether the assays have just as strong predictive potential in a population with lower risk overall. A study that excludes such patients would provide more clinically relevant information. These studies are upcoming.

Including HER2+ and LN+ high-risk patients in the test cohort may bias the analysis and compromise the ability to determine whether the assays have just as strong predictive potential in a population with lower risk overall.

The prohibitive cost of genomic studies is another obstacle associated with these tests. Although clearly dependent on the health care system of the country and on individual health care plans, the value of gene expression assays is still controlled, for the most part, by individual biotechnology companies. Because the tests are not considered entirely routine, as of yet, there is an additional price tag beyond standard tumor processing. We hope that, given the abundance of available tests, competition will eventually drive down the cost of genomic testing.

We still have room to improve on our prognostication efforts. With recent studies revealing the utility of immune-related biomarkers for breast cancer, perhaps, in the near future, levels of tumor-infiltrating lymphocytes or interleukin expression will be incorporated into standard prognostic testing [51]. For now, the currently available assays provide at

least more information than that provided by traditional IHC. They have been shown to provide reproducible results using routinely stored tumor specimens. More important, exploitation of the tumor genetic milieu has proven to be an essential tool for better understanding of tumor biology. As such, the assays enhance our ability to study the nature of each individual tumor; given this information, they serve as a guide for physicians caring for intermediate-risk early stage breast cancer patients. The practice of medicine is both science and art, and ultimately decisions regarding adjuvant chemotherapy will involve a myriad of factors including but not limited to genomic assays.

AUTHOR CONTRIBUTIONS

Conception/Design: Christina Adaniel, Francisco J. Esteva

Provision of study material or patients: Adriana Heguy

Collection and/or assembly of data: Christina Adaniel, Komal Jhaveri

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DISCLOSURES

The authors indicated no financial relationships.

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