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Multi-parametric genomic assays for breast cancer: Time for the next generation?

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Abstract

The Breast Cancer Index appears to perform better than the 21 gene recurrence score in predicting 10-year disease free survival in postmenopausal women with hormone receptor positive lymph node negative early stage breast cancer. This may have implications for clinical use of first generation versus second generation multi-parametric genomic assays.

In this issue of Clinical Cancer Research, Sestak and colleagues (1) compare the performance of a 7 gene prognostic score (Breast Cancer Index, BCI, Biotheranostics, San Diego, CA), with a 21 gene prognostic score (Oncotype Dx, RS, Genomic Health, Redwood City, CA) in a retrospective cohort of 665 patients with hormone receptor positive, lymph node negative early stage breast cancer treated with tamoxifen or anastrozole alone (no chemotherapy) in the Anastrozole, Tamoxifen, Alone or in Combination (ATAC) trial. In particular, these authors compare the prognostic accuracy of both in cases where these assays yielded discordant results. It is noteworthy that the 10 year distant metastasis free survival rate was 89% for this cohort.

The ability to define prognosis and predict response or resistance to systemic therapy for patients with early stage breast cancer is a pressing question given the wealth of data about adjuvant systemic therapy coupled with uncertainty about its application to the individual patient. In an era of mammographic detection the majority of women in the United States are diagnosed with stage 1 or 2 hormone receptor positive breast cancer and face decisions about type and duration of adjuvant endocrine therapy and utility of adjuvant chemotherapy. For many years these decisions were based on clinical factors but a plethora of multigene assays has now come into practice. How and when to utilize these assays are ongoing challenges.

In the Sestak et al analysis 285 of the 665 breast cancers (41.6%) were discordant between RS and BCI. RS re-classified 24.4% of BCI low risk patients into intermediate risk and 3.1% of these patients into high risk. BCI re-classified 21.9% of RS low risk patients into

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intermediate risk and 5.2% of these patients into high risk. Kaplan-Meier analysis was used to determine the accuracy of these reclassifications, and the results were striking. Of the 388 women with low risk RS, 20 women had a high risk BCI score and a median 10-year distant recurrence (DR) risk of 23.3%, and 85 of these women had an intermediate risk BCI with a median 10-year DR risk of 12.2%. Of the 178 woman with an intermediate RS, 34 of these women had a high risk BCI score with a median 10-year DR risk of 27.8%. In contrast, reclassification of BCI risk groups by RS yielded no significant change in 10-year DR risk. How can these results be explained?

The use of RNA expression-based genomic multi-parametric assays for risk stratification in early stage breast cancer grew out of a desire to bring more objectivity to this exercise. Nomograms and algorithms based on clinic-pathologic parameters such as Adjuvant! Online (2) exist, but these algorithms are highly dependent on an accurate subjective assessment of tumor grade.

The first multi-parametric assay to gain clinical acceptance was the 21-gene assay. This assay is comprised of RNA expression analysis of 16 genes selected from the clinical literature of the early 2000s, with the expression of 5 housekeeping genes to normalize RNA expression across multiple samples (3). A regression equation weights the expression of each of the 16 genes, yielding a single continuous score. This score (RS) is further divided into low risk, intermediate risk, and high risk. The 21 gene assay successfully predicted 10-year disease free survival in women with lymph node negative breast cancer treated with tamoxifen with or without chemotherapy in the NSABP 20 trial, and identified a group of women (with high RS) who had a significant benefit from chemotherapy (3).

Close examination of the regression equation used to weight gene expression in the RS revealed that most of the weighting resides in the genes for estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki67 (3). All of these genes can be measured semi-quantitatively at the protein level by immunohistochemistry (IHC). Indeed, several assays, including IHC4 (4) and Magee score (5), use IHC in combination with clinical-pathological factors such as tumor size and Nottingham score to accurately predict the RS as well as 10-year disease recurrence. Additionally, RS does not account for potential mutation of target genes and related transcripts such as ER, with possible alteration of downstream pathways.

Thus, in an effort to further refine multi-parametric genomic classification of recurrence risk in early stage breast cancer, multiple second generation RNA expression-based assays which do not depend as much on ER, PR, HER2, or Ki67 RNA levels have been developed. BCI utilizes a 5 gene genomic grade component comprised of 5 cell cycle genes in combination with a ratio of HOXB13/IL17R (H/I), which are two genes whose expression is related to a functional ER (6), Figure 1. The 70 gene assay (Mammaprint, Agendia, Irvine, CA) is derived from an unsupervised classification of 78 lymph node negative breast cancers into low and high risk of 5-year distant metastasis, and shares only one gene (CD68) with the 21 gene RS (7). The PAM-50 ROR score (Prosigna, Nanostring Technologies, Seattle, WA) is derived from a weighted determination of intrinsic subtype (luminal A, luminal B, HER2 like, and basal like) from 46 genes, in combination with tumor size and a proliferation score of 19 genes (8). All of these assays theoretically measure downstream effects of targets such

as ER, and all of these assays have been shown to have prognostic value (in some cases exceeding that of RS) in determining 10-year disease recurrence rates in retrospective analyses (6,7,8).

Indeed, the measurement of genes other than ER, PR, and HER2 by the BCI assay in the current study may account for the apparent superiority of BCI in selecting out those women at high risk for future recurrence. In particular, BCI, with its estrogen sensitive H/I component, appears to be especially useful in selecting those women with ER positive, lymph node negative breast cancer at higher risk of late recurrence between years 5–10 after diagnosis (9).

So how do we determine which, if any, multi-parametric assay to use in clinical practice and clinical investigation? The first generation 21 gene RS assay appears to perform well in many situations, and recently published prospective data from the TAILOR-Rx trial suggests that women with hormone receptor positive, lymph node negative breast cancer with a RS of 11 or less have an excellent prognosis without chemotherapy (10). A recent presentation of prospective data from the MINDACT trial using the second generation 70 gene assay suggests that women with hormone receptor positive breast cancer, with 0–3 lymph nodes positive, and a low risk 70 gene score, have a 5-year distant metastasis-free survival of greater than 94% with endocrine therapy alone (11). A retrospective analysis of a large Danish cohort of postmenopausal women with hormone receptor positive breast cancer with 0–3 lymph nodes positive and 10 years of follow-up suggests that a cohort of these women with a low risk PAM 50 ROR score had a 10-year disease free survival of 94.3% with endocrine therapy alone (12).

Careful analysis of these studies will be critical as oncologists evaluate the worth of moving from first generation to second generation multi-parametric RNA expression based assays for the assessment of recurrence risk in early stage breast cancer. But wherever possible it will be most useful if prognostic information from any type of assay can be coupled with predictive estimates of the likelihood of benefit from additional therapy.

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Figure 1. Genes comprising two multi-parametric RNA expression assays for breast cancer. Left: Oncotype Dx recurrence score of 16 genes and 5 reference genes for RNA normalization. Right: Breast Cancer Index of 7 genes, split between H:I ratio (2 genes) and Molecular Grade Index (5 genes) with 4 reference genes for RNA normalization. Note the lack of overlap between the gene sets.