Molecular Profiling for Early Stage Breast Cancer Patients

Two assays gain acceptance while awaiting stronger evidence

By Kristine Nally

Traditional prognostic factors for breast cancer including tumor size, histology, lymph node involvement, endocrine receptor (ER) status, and epidermal growth factor receptor (HER2) status have helped to improve treatment regimens and prolong patient survival. Despite these advances, appropriate treatment levels are not always known, and outcomes remain unpredictable even for patients with common, well-defined tumor types. Specifically, endocrine therapy, the most effective adjuvant treatment for ER-positive, lymph node-negative, HER2-negative patients is effective in preventing recurrence in only 50-60% of patients [1]. A better method to determine which patients will respond to endocrine therapy alone and which patients will benefit from additional treatment, such as chemotherapy, would provide significant improvements to outcomes and quality of life for early breast cancer patients.

During the last decade, a large number of molecular profiling studies have been completed. It has now been shown conclusively that breast cancer is a molecularly heterogeneous disease even within distinct groups as defined by traditional prognostic factors. In addition, numerous retrospective studies have found correlations between molecular subtypes and risk of recurrence [1-3]. Most studies have focused on early stage breast cancer with the goal of determining which patients will benefit from neoadjuvant or adjuvant chemotherapy versus patients that can be spared the additional treatment.

In the first round of studies, several microarrays or gene profiles (eg, PAM50 molecular subtypes, MammaPrint 70-Gene Assay, Rotterdam Signature, HOXB13:IL17BR ratio, Oncotype DX 21-Gene RT-PCR Assay) have been successful in demonstrating a higher relative risk of recurrence in specific groups of early stage breast cancer patients [1-3]. All currently published studies are retrospective, and the majority of these studies have demonstrated prognostic ability regardless of treatment. However, some recently published retrospective studies, for both MammaPrint and Oncotype DX, have shown the ability to predict benefits from chemotherapy in specific patient groups and both assays are now part of large, prospective, randomized trials that will measure outcomes when the tests are used to guide treatment decisions.

MammaPrint is a 70-gene molecular signature that was originally developed by researchers at The Netherlands Cancer Institute and is now offered commercially by Agendia. The test currently assesses the risk of recurrence and classifies patients as having a good prognosis (low chance of recurrence) or a poor prognosis (high chance of recurrence). The assay is currently approved by

the Food and Drug Administration (FDA) for for use in lymph node-negative breast cancer patients under 61 years of age with tumors of less than 5 cm, regardless of endocrine receptor status. According to the Agendia website, the MammaPrint assay has been used in 12,000 patients [4].

In the original work, breast tumors from 117 patients, younger than 55 years, with lymph node-negative breast cancer were classified as having either a poor or good prognosis. The results indicated an odds ratio of developing metastatic disease in poor-prognosis patients versus good-prognosis patients of approximately 15 [5]. Several additional studies, by the same group and others, have also shown a statistically significant hazard ratio (HR) when comparing outcomes for patients with a poor versus good gene signature. For example, The Translational Breast International Group (TRANSBIG) completed an independent validation in 302 patients with lymph node-negative disease from five non-Dutch cancer centers [6]. Patients were under 60 years of age with T1 or T2 tumors. The majority of patients did not receive adjuvant chemotherapy. The 70-gene signature was found to be superior in predicting the risk of recurrence and overall survival (OS) when compared to Adjuvant! Online, a software program that uses traditional prognosis factors. The HR for time to distant metastasis was 2.32 (95% CI 1.35 to 4.0) for patients with a good-prognosis signature and 2.79 (95% CI 1.60 to 4.87) for patients with a poor-prognosis signature.

A recent study that assessed 1,637 patients from 7 large data sets across Europe begins to address the need for predictive data [7]. Researchers demonstrated significant improvement in distant disease free survival, from 69% to 88% (HR, 0.28; 95% CI, 0.14-.056; [P<.001]), for patients with a poor-prognosis gene profile that received both endocrine therapy and chemotherapy when compared to patients with a poor-prognosis gene profile that received endocrine therapy alone.

The Oncotype DX assay, available from Genomic Health, is based on a panel of 21 genes (16 cancer-related and 5 reference genes). The test is currently recommended by the American Society of Clinical Oncologists (ASCO) and the National Comprehensive Cancer Network (NCCN) for use in ER-positive, lymph node-negative, HER2-negative breast cancer patients. The assay uses an algorithm to compute a recurrence score (RS) based on the gene expression profile and classifies patients as low-risk, intermediate-risk, or high-risk. According to the Genomic Health website, the test has now been used in over 90,000 clinical cases [8]. Oncotype DX was designed to use paraffin-embedded tumor tissue, a benefit over MammaPrint which requires fresh tissue (frozen or stored in an RNA preservative solution). On the other hand,

Oncotype DX is validated only in ER-positive early stage breast cancer patients, where MammaPrint has been validated in both ER-positive and ER-negative patients.

The first large scale validation of Oncotype DX utilized tumor samples from the National Surgical Adjuvant Breast and Bowel Project Protocol B-14 (NSABP B-14) trial [9]. This trial tested the benefit of 5 years of tamoxifen versus placebo in 2,892 ER-positive, lymph node-negative patients and reported statistically significant improvement in the tamoxifen arm. RT-PCR of the 21-gene profile was completed on 675 samples from patients in the tamoxifen arm. The 10-year distant recurrence rates for the low-risk, intermediate-risk and high-risk groups were 6.8%, 14.3% and 30.5%., with a statistically significant difference in the distant recurrence rate between the low-risk and high-risk groups (P<.001). An additional study using a subset of samples from a Kaiser Permanente case controlled trial reported similar results [10].

The 21-gene Oncotype DX assay has also been evaluated for predictive value. Researchers analyzed 651 tumor samples from ER-positive, node-negative patients that participated in the NSAPB-20 trail [11]. Of the 651 samples, 227 were treated with tamoxifen alone and 424 were treated with chemotherapy (CMF) and tamoxifen. The 21-gene expression profile demonstrated significant chemotherapy benefit in patients with a high-risk RS (mean absolute decrease in 10-year distant recurrence, 28%) and also showed that patients with a low-risk RS received minimal, if any, benefit from chemotherapy (mean absolute decrease in 10-year distant recurrence, 1.1%). Data was not significant in patients with an intermediate RS score.

The results from retrospective studies of both MammaPrint and Oncotype DX have been impressive. However, data from large, prospective, randomized trials remains the gold standard of medical evidence. Two such clinical trials are underway. The TAILORx (Trial Assigning IndividuaLized Options for Treatment) trial will perform the Oncotype DX assay on ER-positive, lymph-node negative patients in the United States. Patients with a low RS (<11) will receive endocrine therapy alone, patients with a high RS (>25) will receive both endocrine therapy and chemotherapy. Patients with an intermediate RS (between 11 and 25) will be randomized to endocrine therapy alone or endocrine therapy and chemotherapy. In the MINDACT (Microarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy) trial, patients will be evaluated by both Adjuvant! Online and MammaPrint. If both methods indicate a low risk, patients will receive endocrine therapy alone and when both methods indicate a high risk, patients will receive chemotherapy and endocrine therapy. When the two evaluation methods are discordant, the patient will be randomized to receive endocrine therapy alone or endocrine therapy and chemotherapy.

While awaiting conclusive data, both MammaPrint and Oncotype DX molecular profiling assays are being used in clinical practice. In addition to the specific FDA, ASCO, and NCCN endorsements mentioned above, the general use of a "validated, multigene-profiling assay, when available" was endorsed at the March 2009 St. Galan International Expert Consensus meeting on the treatment of early stage breast cancer [12]. However, Evaluation of Genomic Applications in Practice and Prevention (EGAPP) guidelines published in January 2009 found the evidence for the use of molecular assays in early stage breast cancer to be insufficient [13].

In conclusion, the promise of personalized medicine is proving to be more difficult than originally assumed and progress is being made incrementally. Both commercially available tests for early stage breast cancer, MammaPrint and Oncotype DX, can provide prognostic, but not predictive, information and should be used in addition to traditional clinical/pathological data to decide the most appropriate course of treatment.

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