# Clinical Utility of Emerging Molecular Diagnostics in Breast Cancer

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Abstract

Molecular diagnostic tests are increasingly used in the clinic to tailor therapy to the molecular characteristics of a cancer. In early stage estrogen receptor (ER)-positive cancer, the 21-gene recurrence score assay, as well as other gene expression signature-based tests are routinely used to assist in identifying patients for adjuvant chemotherapy. A new generation of tests has also been developed for ER-positive cancers to estimate the risk of late recurrence and benefit from extended adjuvant endocrine therapy and therefore identify patients who require 10 years of endocrine therapy. Among triple-negative breast cancers (TNBC), there is increasing evidence that patients with germline BRCA mutant cancers are highly sensitive to platinum drugs, and inclusion of this agent with an anthracycline- and taxane-based regimen might improve their outcome. Other proposed "BRCA-ness" markers are also being tested in the clinic. Extensive immune cell infiltration (lymphocyte predominant breast cancer) that can be detected by counting tumor-infiltrating lymphocytes or measuring immune-specific gene expressions is an increasingly recognized favorable prognostic marker that also predicts greater chemotherapy sensitivity in TNBC and high-risk, ER-positive cancers. The clinical utility of immune markers is yet to be defined, but their importance will likely grow as immunotherapies are entering the clinic. In HER2-positive cancer, several markers demonstrated statistically significant associations with response to HER2-targeted therapies, but their predictive values to select one therapy over another or to omit HER2-targeted therapies are modest and have little clinical utility. Molecular target profiling of metastatic cancers is increasingly performed to determine clinical trial eligibility for targeted drugs and to identify potentially druggable alterations. Anecdotal examples demonstrate benefit from off-label use of targeted therapies in some patients with a matching molecular abnormality, but the clinical utility of this strategy is still under study in several ongoing clinical trials.

**Key words:** breast cancer, molecular target profiling, recurrence score, HER2, estrogen receptor, metastatic cancers, triple-negative breast cancer

## Introduction

Clinically useful molecular diagnostic tests enable more selective use of therapies and therefore could lead to more cost-effective care and spare patients from potential side effects of ineffective therapies. In breast cancer, several diagnostic tests have emerged that meet criteria for clinical use, and many other tests are currently being investigated in clinical studies to assess their clinical value.

## Estrogen Receptor-Positive Cancer

For estrogen receptor (ER)-positive early-stage breast cancer, molecular tests are available to assist in answering two important questions: who benefits from adjuvant chemotherapy, and who remains at risk for late recurrence despite 5-years of adjuvant endocrine therapy and therefore may require extended adjuvant endocrine therapy? The OncotypeDX 21-gene recurrence score, the Prosigna PAM50-risk-of-recurrence score, the MammaPrint gene signature, and the Breast Cancer Index each represent standardized, commercially available mRNA expression-based molecular diagnostic tests in the US that can be used to identify patients who are the most likely to benefit from adjuvant chemotherapy through measuring the expression of estrogen signaling and proliferation-related genes. The predictive utility of these tests is derived from their ability to simultaneously identify cancers that are high-risk for recurrence and also have sensitivity to chemotherapy.[1] The predictive value of each of these tests is supported by substantial evidence, and all major breast cancer practice guidelines support the use of multigene diagnostic assays, in conjunction with other clinical pathological parameters, to help select patients for adjuvant chemotherapy. However, when more than one assay is applied to the same cancer, they can yield discordant prediction results as often as 20 to 30% of the time, particularly for borderline risk categories.[2,3]

The sample-level concordance between test results is modest because they rely on different genes, algorithms, and platforms and currently it remains unknown how to handle discordant results.[3] Several studies have demonstrated the cost effectiveness of these assays but the impact of test results on adjuvant chemotherapy use depends on the patient population that is being tested.[4-6] In clinically high-risk patient populations (e.g. node-positive or >1 cm) chemotherapy use tends to decrease,

while in the low-risk populations (e.g. node-negative and <1 cm) chemotherapy use increases among patients who had testing compared to those who were not tested.[7]

Overall, at least half of all metastatic recurrences of ER-positive breast cancers occur after 5 years. Several randomized clinical trials reported improved recurrence-free survival (RFS) with extended endocrine treatment using tamoxifen or an aromatase inhibitor (ATLAS, MA.17, aTTom, NASBP-B33, ABCSG6a) and one study (ATLAS) also showed improved overall survival with 10 years of tamoxifen compared to 5 years.[8] However, the absolute benefit is small (2 to 3% improvement in RFS) and extended endocrine therapy implies financial costs and increased risk for adverse events.[9] For example, in the ATLAS trial, 340 patients needed to be treated to prevent six deaths from breast cancer while the treatment caused approximately three extra endometrial cancers and one extra pulmonary embolism. Several emerging molecular tests can identify patients who are at risk for late recurrence including the Breast Cancer Index (BCI), the Prosigna PAM50 Risk of Recurrence (ROR) score, and the EndoPredict test (available in Europe).[10-14] Typically, patients with high proliferation scores and high estrogen-related gene expression or low proliferation scores and low estrogen-related gene expression are at the greatest risk for late recurrences.[13] Unlike the other tests in this space, the BCI has also been shown to predict benefit from extended endocrine therapy in archived samples of the MA17 trial that compared 5 years of tamoxifen with 5 years of tamoxifen followed by 5 years of letrozole and therefore may be particularly helpful to identify patients for extended endocrine therapy.[11] A study that examined the impact of BCI results on medical decision making showed that physician recommendations for extended endocrine therapy changed for 25% of patients after considering BCI results, with a net decrease in extended endocrine therapy.[12] Testing also led to increased patient satisfaction, decreased decision conflict and anxiety, and resulted in fewer patients wanting to receive extended therapy.[12]

Efforts are also under way to develop tertiary risk predictors that could measure residual risk of recurrence after completion of adequate adjuvant endocrine and chemotherapies. Current molecular diagnostic tests such OncotypeDx, Prosigna PAM50-ROR score, BCI, etc. identify patients who are high risk for recurrence with endocrine therapy alone and therefore good candidates for adjuvant chemotherapy. However, many but not all of these patients will revert to low-risk status after completing chemotherapy. Identifying those who remain at high risk for recurrence despite the best current therapies is important because it defines the ideal patient population for adjuvant clinical trials. One study demonstrated the feasibility of this approach by re-stratifying OncotypeDx high-risk patients who all received endocrine plus chemotherapy, into good versus poor prognosis groups with 5-year RFS of 95% and 76%, respectively, using a multigene signature.[15]

### Triple-Negative Breast Cancer

No clinically meaningful molecular prognostic tests emerged for triple-negative breast cancer (TNBC) despite extensive research. The prognostic assays that are used in ER-positive cancers invariably categorize ER-negative tumors as high risk. Immune cell infiltration is currently emerging as a prognostic and chemotherapy predictive marker in TNBC. High tumor-infiltrating lymphocyte (TIL) count or high expression of immune gene signatures are consistently associated with better survival.[16-19] Additionally, neoadjuvant studies demonstrated significantly higher pathological complete response (pCR) rates among immune-rich compared to immune-poor TNBC indicating a chemotherapy response predictive role.[16,20-22] An international guideline was recently published to standardize TIL assessment, preparing the stage for introducing this prognostic variable into routine pathology reporting.[23]

A substantial amount of preclinical data suggest the many TNBC harbor DNA repair deficiencies, and therefore drugs that induce DNA damage may be particularly effective. Clinical trials in the neoadjuvant and metastatic treatment settings demonstrated that platinum drugs have activity in TNBC, but overall these drugs are not more effective than other active agents such as taxanes.[24-28] In an attempt to identify the subset of patients with TNBC that may be particularly sensitive to DNA-damaging agents, several molecular tests are being developed that quantify DNA repair deficiency. One of the most extensively studied assays is the Homologous Recombination Deficiency (HRD) score, which combines three different consequences of DNA repair defect on DNA structure including loss of heterozygosity, telomeric allelic imbalance, and large-scale state transition metrics, into a single HRD score.[29-32] This score is highly correlated with the presence of germline BRCA-1 and -2 mutations and BRCA promotor methylation but the score can also be high in BRCA wild type and non-methylated cases.

Studies have shown that a high score is predictive of response to platinum therapy in TNBC and ovarian cancer, but it also seems to be associated with more general chemotherapy sensitivity including anthracycline and taxane-based regimens.[29] Studies have shown that a high score is predictive of response to platinum therapy in TNBC and ovarian cancer, but it also seems to be associated with more general chemotherapy sensitivity including anthracycline and taxane based regimens[29]. A randomized phase III trial that compared single-agent carboplatin with docetaxel as first-line therapy for metastatic TNBC revealed that both drugs were equally effective and a high HRD score could not define the subpopulation that was more sensitive to platinum than to the docetaxel. However, in the BRCA-1 and -2 positive population, carboplatin showed a significantly increased activity compared to docetaxel.[24] These results are consistent with the reported high activity of Cisplatin in BRCA-1 positive stage I to III breast cancer in neoadjuvant trials.[33] Several correlative studies are

**TABLE.** Molecular Diagnostic Tests Most Commonly Available in the Clinic (other than ER, PR and HER2) and Their Endorsement by Practice Guidelines and the US FDA

| Assay | Endorsed by | Endorsed Use | Not Endorsed, But Variable Level of Data Supports Use |
|---|---|---|---|
| OncotypeDX 21-gene recurrence score | ASCO, NCCN, St Galen | ER+, estimate risk of recurrence, select for adjuvant chemotherapy | Predict greater chemotherapy sensitivity in ER+ cancer; predict recurrence after 5 years |
| Prosigna PAM-50 risk of recurrence score | ASCO, NCCN, St Galen | ER+, estimate risk of recurrence, select for adjuvant chemotherapy | Predict recurrence after 5 years; predict greater chemotherapy sensitivity in ER+ cancer |
| Breast Cancer Index (BCI) | ASCO, St Galen | ER+, estimate risk of recurrence, select for adjuvant chemotherapy | Predict recurrence after 5 years; predict benefit from extended endocrine therapy |
| EndoPredict | ASCO, Galen | ER+, estimate risk of recurrence, select for adjuvant chemotherapy | Predict recurrence after 5 years |
| MammaPrint | St Galen | ER+, estimate risk of recurrence, select for adjuvant chemotherapy | Predict greater chemotherapy sensitivity in ER+ cancer |
| Ki67 immunohistochemistry | St Galen | ER+, estimate risk of recurrence, select for adjuvant chemotherapy | Predict greater chemotherapy sensitivity in ER+ cancer |
| Tumor Infiltrating Lymphocyte (TIL) count | None | N/A | Predict prognosis in TNBC, HER2+ cancers |
| HRD homologous recombination deficiency assay | None | N/A | Predict greater sensitivity to DNA-damaging chemotherapy and PARP inhibitors |
| Germ-line BRCA mutation as treatment response marker | St Galen | TNB—Consider platinum drug for therapy | Predict greater sensitivity to DNA-damaging chemotherapy and PARP inhibitors |
| Foundation One targeted next-generation gene sequencing | None | N/A | Molecular target profiling of metastatic cancer for clinical trial triaging or off-label use of targeted drugs |

ASCO, American Society of Clinical Oncology; NCCN, National Comprehensive Cancer Network; St Galen indicates St Galen Breast Cancer Practice guidelines; TNBC, triple negative breast cancer; PARP, Poly ADP-ribose polymerase.

under way to further define the predictive value of the HRD score as well as other similar DNA damage signatures, and based on the currently available data, germline BRCA status appears to be the best predictor of "above average" sensitivity to platinum drugs in breast cancer.

## HER2-Positive Breast Cancer

Despite extensive clinical efforts and some impressive preclinical models, there are no currently clinically useful molecular predictors to select one HER2 targeted therapy over another or to recommend against using HER2 targeted therapy in HER2-

positive breast cancers. Several neoadjuvant and metastatic trials have demonstrated that mutations in the phosphatidylinositol 3-kinase catalytic subunit (PIK3CA) and extensive immune cell infiltration are negative and positive predictors of pathologic complete response to HER2 targeted therapies, respectively.[22,34-36] However, their positive and negative predictive values are modest and they also appear to interact with prognosis independent of treatment response, which limits their clinical utility. Other molecular predictors of response have also been proposed based on biological insights into the mechanisms of resistance to HER2 targeted therapies including altered expression and structure of the HER2 receptor, constitutive activation of the downstream signaling pathways, and switching to alternative growth and survival pathways, but none of these have been validated consistently in clinical studies.

## Molecular Target Profiling of Metastatic Breast Cancer

Molecular target profiling of metastatic cancers is increasingly performed in the clinic to identify potentially druggable alterations. Many clinical trials require testing for particular molecular defects that may sensitize cancers to an investigational drug as part of their eligibility criteria, and anecdotal case histories demonstrate benefit from off-label use of targeted therapies in some patients with a matching molecular abnormality. There are several CLIA-certified academic and commercial molecular pathology laboratories (eg, Foundation Medicine, Caris Life Sciences, Paradigm PCDx) that provide targeted next- generation sequencing (NGS) or a combination of NGS and other methods to perform drug target profiling. The analytical validity of the commercial assays is often published but the clinical validity (ie, response predictive value) and clinical utility (ie, demonstration of improved patient outcome because of using a test) in solid tumors have not been established beyond a few mutations in melanoma (BRAF), gastrointestinal stromal cell tumors (KIT/PDGFRA), and lung (ALK/AML4, EGFR) and colorectal cancers (KRAS).

A central premise behind cancer target profiling is that tumors that share the same driver mutation will respond to a corresponding targeted drug regardless of histologic subtype whereas cancers without the mutation will not. Laboratory experiments and a few clinical success stories support this hypothesis (eg, trastuzumab activity in HER2 amplified gastric cancer), but there are also numerous clinical studies indicating that the target function of a given gene or mutation is molecular context- and cancer type-dependent (eg, low tamoxifen activity in ER-positive ovarian cancer, lack of vemurafenib activity in BRAF mutant in colon cancer).[37,38] Furthermore, each cancer harbors a large number of potential functionally important genomic alterations, which makes it difficult to designate a single abnormality as the driver event.[39] Comparative genomic hybridization studies showed that the genome of an average breast cancer contains 76 large-scale copy number alterations; exome sequencing studies showed at least 30 to 80 deleterious single nucleotide mutations[40,41] and these somatic events arise in the background of several thousand high functional impact germline single-nucleotide polymorphisms that each individual carries.[42] This complexity of the cancer genome, along with the numerous redundant and compensatory biological pathways, may explain the modest correlation between the activity of many targeted drugs and alterations in their corresponding targets. For example, there is only modest correlation between PI3K mutation status and benefit from mTOR or PI3K inhibitors.[43-46] There is also little correlation between CDK 4/6 amplification and benefit from CDK 4/6 inhibitors.[47,48] Despite the mixed success, systematic testing of this concept in prospective clinical trials is important. Several nationwide industry-sponsored clinical trials including the Signature trial by Novartis (www.signaturetrial.com), the My Pathway trial by Genentech (http://clinicaltrials.gov/show/NCT02091141), the NCI-MATCH (www.ctsu.org), and the ASCO TAPUR (TAPUR@asco.org) trial, are currently open for patients with breast cancer to determine the clinical value of a broad spectrum of molecularly targeted therapies and the clinical utility of cancer target profiling.

## Conclusions

In summary, the past decade of biomarker research has led to the introduction of several multigene prognostic tests that are now routinely used in clinical decision making to assist the selection of patients for adjuvant chemotherapy, and several other tests have emerged with potential clinical utility. In recognition of these advances and to help physicians use molecular diagnostics wisely, ASCO has recently issued new recommendations for biomarker use in early-stage breast cancer.[49]

The guideline summarizes the type and quality of evidence that supports the recommendations, which are further qualified by levels of strength. The ASCO guideline states that the clinician either "may use" or "should not use" a particular assay. The ASCO recommendations are listed in the TABLE, along with the positions of other practice guidelines (NCCN and St Galen) on the same tests. While the ASCO guidelines provide practical clarity, they do not do full justice for the available evidence for some uses of these tests. For example, the recommendation that genomic prognostic tests developed for ER-positive cancer (OncotypeDx, etc.) should not be used in TNBC is based on consistent and broad evidence that demonstrated no clinically meaningful risk stratification function in this disease setting. On the other hand, the recommendation that these tests should not be used in patients with one or two positive nodes is based on well-reasoned caution even though multiple studies consistently demonstrated continued independent, prognostic function in node-positive patients and some of the tests even provide nodal status-adjusted risk estimates. Similarly, the guideline

recommends against using any of the tests endorsed for risk prediction in ER-positive cancer to guide selection of extended adjuvant therapy, despite multiple publications supporting their ability to predict late recurrences, and in the case of at last one assay, there is a data to suggest that those at high risk for late recurrence also benefitted from extended endocrine therapy. Practicing oncologists often encounter clinical scenarios when test results with some degree of uncertainty still provide helpful information when considered together with other (often similarly uncertain) disease-related variables. Several emerging tests with proven analytical and clinical validly but yet uncertain clinical utility occupy that space.

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