Advances in the Molecular Analysis of Breast Cancer: Pathway Toward Personalized Medicine

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**Background:** Breast cancer is a heterogeneous disease that encompasses a wide range of clinical behaviors and histological and molecular variants. It is the most common type of cancer affecting women worldwide and is the second leading cause of cancer death.

**Methods:** A comprehensive literature search was performed to explore the advances in molecular medicine related to the diagnosis and treatment of breast cancer.

**Results:** During the last few decades, advances in molecular medicine have changed the landscape of cancer treatment as new molecular tests complement and, in many instances, exceed traditional methods for determining patient prognosis and response to treatment options. Personalized medicine is becoming the standard of care around the world. Developments in molecular profiling, genomic analysis, and the discovery of targeted drug therapies have significantly improved patient survival rates and quality of life.

**Conclusions:** This review highlights what pathologists need to know about current molecular tests for classification and prognostic/predictive assessment of breast carcinoma as well as their role as part of the medical team.

**Introduction**

For years the diagnosis and classification of breast cancer has been based on clinicopathological features such as tumor type and size, lymph node status, and histological grade. However, during the last few decades, significant advances have taken place in this area as we approach the era of personalized medicine.

Breast cancer comprises a heterogeneous group of tumors that significantly vary in their responses to treatment, presentation, and biology. For example, histologically similar tumors may have different clinical behavior and responses to treatment. Significant advances in personalized treatment during the last decade have been due to genomic analysis, which allows for the molecular study and classification of tumors and gives rise to the availability of biological targeted drugs such as trastuzumab for human epidermal growth factor receptor 2 (HER2)–positive breast cancer. However, the biological heterogeneity of tumors continues to be problematic because only a subset of patients with a particular type of tumor will benefit or respond to targeted treatments. In addition, most commercially available assays are designed to determine prognostic and predictive information in early-stage carcinoma and, thus, offer little clinical value to patients with advanced stage or aggressive variants of breast cancer. Therefore, it is important

Molecularly targeted therapy may offer more tailored, personalized treatments for patients with breast cancer.
to continue developing tests that can predict the risk of cancer recurrence as well as which patients will respond to specific therapeutic measures.

**Evaluation Using Traditional Methods**
Historically, breast cancer has been classified using histological grade. The first attempts to grade breast cancer based on histology were initiated by Grecnough in 1925, followed by Patey and Scarff in 1928, Haagensen in 1933, and Bloom and Richardson in 1957. Elston's modified Bloom–Richardson grading system (also called the Nottingham system) is now used to grade breast cancer. However, interobserver variability and less-than-ideal reproducibility exist among pathologists regarding tumor grading. Nevertheless, tumor grading plays a role in the diagnosis and management of breast cancer because its value as an independent prognostic factor for overall survival rates in spite of tumor size and nodal status has been proven in numerous studies.

In addition to tumor grade, pathologists have traditionally classified breast carcinoma into histological types. The general consensus is that select types of carcinoma of the breast are associated with distinctive, biological characteristics related to clinical behavior. However, the classification and diagnostic criteria for each tumor type have also varied throughout the years. Select special-type carcinomas are uncommon; thus, they cannot always be included in long-term or molecular studies.

**Estrogen and Progesterone Receptors**
The estrogen receptor (ER) and progesterone receptor (PR) are prototypical tumor markers that have an immediate impact on systemic treatment decisions for patients with breast cancer. ER and PR are more commonly positive in low- to intermediate-grade tumors and in postmenopausal women. They are considered weak prognostic markers but strong predictive factors of tumor response to hormonal therapy (eg, tamoxifen). PR is also an independent predictor of response because ER- and PR-positive patients have better responses to treatment than ER-positive, PR-negative patients.

ER and PR are measured using immunohistochemistry (IHC), which is the preferred method for measurement due to its wide availability and ease of use. However, it is estimated that nearly 20% of all ER and PR testing may not be accurate, and concerns regarding current testing, interlaboratory reproducibility, and possible false-negative or false-positive results exist. Several factors influence the accurate testing of ER and PR, including specimen type, fixation type and time, tissue decalcification, automated compared with manual procedure, antibody selection, threshold for positivity, and quality assurance and control. For optimal ER and PR testing, tissue must be sectioned at 5-mm intervals and fixed in a sufficient amount of 10% buffered formalin for at least 6 hours but no more than 72 hours. Cold ischemic time should be for no more than 1 hour. In addition, if nuclear staining is present in 1% or more tumor cells, then the results are positive. Every PR and ER IHC assay should include positive and negative controls.

**Human Epidermal Growth Factor Receptor 2**
The HER2 oncogenic protein is a transmembrane glycoprotein member of the HER family encoded by ERBB2. HER2 is expressed at low levels in several normal epithelia, including the breast. HER2 amplification and the accompanying protein overexpression occur in approximately 15% to 20% of breast cancers. HER2 overexpression, gene amplification, or both are independent prognostic markers of clinical outcomes. HER2 is also a predictive factor of tumor response to chemotherapeutic agents. Its status is typically evaluated to determine patient eligibility for anti-HER2 therapy. When used as monotherapy or in combination with other drugs or chemotherapeutic agents, HER2-targeted drugs have significantly improved survival and response rates.

HER2 status can be determined in formalin-fixed, paraffin-embedded (FFPE) tissue by assessing protein expression on the membrane of the tumor cells using IHC or by assessing the number of HER2 copies using in situ hybridization. For adequate testing, the fixation process should be initiated within 1 hour of tissue removal and the total fixation time should range from a minimum of 6 hours to a maximum of 72 hours. By IHC, scores of 0 and 1+ are considered to be negative, a score of 2+ is considered to be positive, and a score of 3+ constitutes a “gray zone” in which testing with an alternative methodology is necessary.

In situ hybridization methods include fluorescence in situ hybridization, chromogenic in situ hybridization, dual in situ hybridization, and silver-enhanced in situ hybridization. Some assays use a single probe to determine the number of HER2 copies; however, most assays also include a chromosome enumeration probe (CEP17) chromosome 17. Results are reported as follows:

- Not amplified; ratio < 2.0 with an average number of HER2 copies < 4.0 signals/cell
- Amplified ratio ≥ 2.0 or an average HER2 copy number ≥ 6.0 signals/cell
- Equivocal < 2.0 with an average HER2 copy number ≥ 4.0 and < 6.0 signals/cell

Several factors influence the accuracy of HER2 testing and tumor response to therapeutic agents. In the metastatic setting, response rates to trastuzumab are below 50%, and many patients initially responding to trastuzumab therapy go on to develop
tumor recurrence and drug resistance. Although variable responses to trastuzumab may not all be related to inaccuracies in testing, the standardization of testing methodologies across laboratories is problematic; moreover, approximately 20% of HER2 testing may be inaccurate.

The most common factors that affect results include prolonged fixation in formalin, fixation in nonformalin fixatives, and tissue decalcification that may degrade DNA. Coamplification of CEP17 and HER2 can occur in breast cancer, causing miscalculation of the HER2:CEP17 ratio and, thus, underreporting of HER2 amplification. In addition, some tumors may demonstrate intratumoral heterogeneity for HER2 amplification. Although this definition has been challenged, HER2 tumor heterogeneity has been defined as a tumor that has at least 5% but no more than 50% of nonclustered tumor nuclei and a HER2:CEP17 ratio higher than 2.2. HER2 tumor heterogeneity may range between 5% and 15% of total cases tested and tumor heterogeneity may be more frequent (up to 27%) in breast carcinomas with an equivocal (2+) HER2 score.

Concordance between HER2 IHC (protein expression) and in situ hybridization (gene amplification) is required in at least 95% of cases. Among more than 1,500 patients whom they centrally screened, Perez et al found discordant results in approximately 4%. These findings may be due to amplification without overexpression, marked intratumoral heterogeneity, or protein overexpression without amplification. Response to anti-HER2 therapy in these cases is unknown.

**Human Epidermal Growth Factor Receptor 2 Testing Using Other Methodologies**

The HERmark (Monogram Biosciences, South San Francisco, California) assay uses a dual antibody approach to make quantitative measurements of HER2 content in FFPE tissue based on the VeraTag Technology platform (Monogram Biosciences). Compared with single antibody-based IHC methods, the dual antibody approach increases rates of specificity and sensitivity. HER2:HER2 homodimers and HER2 total protein are measured by the assay. Current testing methods identify patients eligible for trastuzumab. Alternatively, HERmark can distinguish subpopulations of patients likely to have different clinical outcomes; for example, those with higher levels of expression may have better outcomes than those with lower expression levels. Although the data is limited, studies suggested that compared with other marketed tests, HERmark can more accurately identify patients likely to respond to trastuzumab.

**Other Prognostic and Predictive Methods**

Many women diagnosed with early-stage, ER-positive, HER2-negative breast cancer receive systemic chemotherapy in addition to hormonal therapy following surgery to increase the likelihood of cure and reduce the risk of recurrence. However, it is possible that many of these women could be spared from the toxic adverse side effects of systemic chemotherapy if they were accurately stratified into groups that may or may not benefit from adjuvant chemotherapy.

Although it is not a molecular diagnostic test, Adjuvant! Online (www.adjuvantonline.com) is an Internet-based tool whose purpose is to help health care professionals and patients with early breast cancer review the benefits of adjuvant therapy following surgery, whether it is hormonal therapy, chemotherapy, or both. This online tool uses information such as estimates of comorbidity, tumor staging and characteristics (eg, ER status, size of tumor, number of positive axillary nodes), menopausal status, and age to provide baseline prognostic estimates of 10-year outcomes (with and without adjuvant systemic therapy). Most of the prognostic information is based on data from the Surveillance, Epidemiology, and End Results program, and projections of the efficacy rates of adjuvant therapy are based on data from the Early Breast Cancer Trials’ Collaborative Group.

The Adjuvant! Online tool estimates the efficacy of endocrine therapy when used as monotherapy or in combination with systemic chemotherapy to determine likely outcomes. The tool can also model overall survival and disease-free survival rates as well as improvements seen in clinical trials. However, the Adjuvant! Online tool tends to overestimate the risk of recurrence in some patients.

The IHC prognostic model IHC4 uses the quantitative values of 4 standard IHC assays (ER, PR, HER2, and Ki67). The test was developed using data from a cohort of 1,125 ER-positive patients who did not receive adjuvant chemotherapy, had a genomic health Oncotype (Genomic Health, Redwood City, California) Recurrence Score, and had adequate tissue for the 4 IHC measurements (Arimidex, Tamoxifen, Alone or in Combination (ATAC) trial). The test was further validated using data from another cohort of 786 ER-positive patients. To strengthen the IHC4 value, researchers combined it with clinicopathological parameters such as tumor grade, size, nodal burden, patient age, and type of endocrine treatment (aromatase inhibitors or tamoxifen), thus creating the IHC4 + clinical (IHC4+C) score. The IHC4+C score helps predict the residual risk of distant recurrence at 9 years in postmenopausal women with node-negative, hormone receptor-positive disease treated with 5 years of adjuvant endocrine therapy independent of the type of endocrine therapy; however, the test...
cannot provide predictive information regarding drug of choice. Other researchers have concluded that the IHC4+C score provides additional prognostic information in this population that is at least as informative as Oncotype DX (Genomic Health). It provides a less costly and readily available methodology because 3 of the included IHC measurements are routinely performed in all patients diagnosed with breast cancer. However, variation in tissue handling, laboratory testing, and reporting of IHC tests hinders its generalized clinical use.

Barton et al compared the IHC4+C score with the Adjuvant! Online tool and found that the IHC4+C score additionally stratified patients according to their residual risk of distant recurrence among those already determined by Adjuvant! Online as being of intermediate risk. The IHC4+C score decreased the level of risk in more than 50% of individuals defined as having intermediate risk per the Adjuvant! Online low risk, thus sparing some patients from adjuvant chemotherapy. In addition, Barton et al inferred that the IHC4+C score may have clinical utility in individuals labeled as high risk by Adjuvant! Online because the IHC4+C score downgraded almost one-half of these patients to the intermediate risk group and a select few were downgraded to the low-risk group. No additional usefulness of the IHC4+C score was seen in patients classified as low risk by the Adjuvant! Online tool, thus, this online tool may be sufficient for treatment decisions in these patients.

Gene-Expression Profiling

Gene-expression profiling has been used for more than 10 years to develop tests so that accurate and personalized clinical outcomes can be better predicted when compared with traditional pathological and clinical standards. In 2000, Perou et al described for the first time a molecular classification system for breast carcinoma, identifying 4 major molecular subtypes: ER-positive/luminal-like, basal-like, ERBB2-positive (HER2-enriched), and normal breast-like. Subsequent studies redefined the intrinsic molecular classification, resulting in a subdivision of the luminal type into types A and B. Luminal type A cancers have a low morphological grade and are predominantly positive for ER, whereas luminal type B cancers are also predominantly positive for ER, often have a high morphological grade, and may sometimes express low levels of hormone receptors. Amplification and high expression of ERBB2, along with several other genes of the ERBB2 amplicon, characterize HER2-enriched cancers. Most (but not all) basal-like tumors correspond to ER-, PR-, and HER2-negative tumors (Table 1). The normal breast-like subtype has not been reproducibly defined and is thought to be an artifact of having too few tumor cells and a background of normal breast tissue in the sample.

Although this classification gained acceptance, the initial testing methodology used messenger ribonucleic acid (mRNA) expression analysis in fresh frozen tissue, thus hampering its introduction into clinical practice. To identify subtypes using standard FFPE tissue specimens, other alternatives have been sought. Although intrinsic subtypes can be approximated using IHC stains, such as ER, PR, HER2, Ki67, epidermal growth factor receptor, and cytokeratins 5/6, 7, 8, 17, 18, and 19, the use of IHC for this purpose has not gained wide acceptance because the concordance with other molecular methods is not perfect and because of the complexity introduced when using multiple IHC markers on limited amounts of tissue.

Gene-expression profiling helps to identify genes with potential to be used as a molecular signature in guiding therapy and predicting patient prognosis. Popular breast cancer multigene predictor test platforms include IHC, fluorescence in situ hybridization, reverse transcriptase–polymerase chain reaction (RT-PCR),

![Table 1. — Summary of Clinical and Pathological Features of Main Intrinsic and Molecular Breast Cancer Subtypes](image)

<table>
<thead>
<tr>
<th>Intrinsic Type</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>HER2 Enriched</th>
<th>Basal Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological Grade</td>
<td>Low to intermediate</td>
<td>Intermediate to high</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Breast Carcinomas, %</td>
<td>40</td>
<td>20</td>
<td>20–30</td>
<td>~ 15</td>
</tr>
<tr>
<td>Most Common Marker Results</td>
<td>ER positive</td>
<td>PR positive</td>
<td>ER (weaker) positive</td>
<td>ER negative</td>
</tr>
<tr>
<td></td>
<td>HER negative</td>
<td>PR positive or negative</td>
<td>HER2 positive or negative</td>
<td>HER2 positive</td>
</tr>
<tr>
<td></td>
<td>Low Ki67</td>
<td>Higher Ki67</td>
<td>Higher Ki67</td>
<td></td>
</tr>
<tr>
<td>Prognosis</td>
<td>Good</td>
<td>Intermediate</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Mutations in TP53</td>
<td>High risk of relapse</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Targeted Treatment</td>
<td>Hormonal therapy</td>
<td>Hormonal therapy</td>
<td>HER2-targeted therapies (eg, trastuzumab)</td>
<td>No targeted treatment options</td>
</tr>
<tr>
<td>Tumor Histology</td>
<td>CK = cytokeratin, EGFR = epidermal growth factor receptor, HER2 = human epidermal growth factor receptor 2.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
and microarray technology. Several tests are commercially available and many more are in development (Table 2). Some of the most clinically used methodologies will be discussed.

**Immunohistochemistry-Based Multigene Assay**

Mammostrat (Clarient, Aliso Viejo, California) can help health care professionals make decisions regarding the use of chemotherapy as well as endocrine therapy in patients with ER-positive, early-stage breast cancer. Mammostrat uses IHC to evaluate the gene expression of p53, SLC7A5, CEACAM5, NDRG1, and HTP9C, which have been selected from 700 gene targets in 3 individual cohorts, and has been comprehensively and clinically validated. The biomarkers are then analyzed using an algorithm that assesses risk for cancer recurrence, which is reported as low (7.6% likelihood of distant recurrence during a 10-year period), moderate (16.3% likelihood of distant recurrence during a 10-year period), or high (20.9% likelihood of distant recurrence during a 10-year period). This calculated risk of recurrence is independent of grade, lymph node status, and stage. In addition, the test appears to identify biological drivers of disease relapse that complement traditional pathological findings, such as lymph node status, tumor grade and size, and other biological markers (eg, HER2).

**Reverse Transcriptase–Polymerase Chain Reaction–Based Multigene Assays**

Oncotype DX is a prognostic, predictive, 21-gene profile, real-time RT-PCR assay performed by a central laboratory using FFPE samples of breast cancer. In individuals with ER-positive, HER2-negative, lymph node–negative carcinoma, the assay is used to determine their 10-year risk for disease recurrence, thus assigning them a recurrence score of low risk (< 18), intermediate risk (18–30), or high risk (≥ 31). Oncotype DX was developed after researchers identified and analyzed 250 candidate genes from 447 participants from 3 separate studies, eventually leading to its 21-gene profile that includes 5 reference genes as internal controls and 16 genes related to cancer. For the assay, the proliferation and ER pathways followed by the HER2 pathway play the most influential role in the calculation of the recurrence score.

Current guidelines advise oncologists to withhold chemotherapy in patients with low recurrence scores but to offer treatment to those with high recurrence scores. Patients with an intermediate recurrence score constitute a “gray zone.” To further clarify the risk of recurrence and benefit from chemotherapy in such patients, the Trial Assigning Individualized Options for Treatment (TAILORx trial) was initiated and is ongoing.

In addition to risk of recurrence, Oncotype DX also appears to predict chemotherapy benefits. In 2 large studies, lack of benefit from chemotherapy was associated with lower recurrence scores, and greater benefit from adjuvant therapy was associated with higher recurrence scores.

Oncotype DX is included in treatment guidelines from the American Society of Clinical Oncology and National Comprehensive Cancer Network. How-

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Monogram Biosciences (South San Francisco, CA)</th>
<th>Clarient Diagnostic Services (Aliso Viejo, CA)</th>
<th>Genomic Health (Redwood City, CA)</th>
<th>NanoString Technologies (Seattle, WA)</th>
<th>Agendia (Irvine, CA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Sample</td>
<td>FFPE</td>
<td>FFPE</td>
<td>FFPE</td>
<td>FFPE</td>
<td>Fresh, frozen, or FFPE</td>
</tr>
<tr>
<td>Type of Assay</td>
<td>Quantitative measurements of ERBB2</td>
<td>21-gene recurrence score</td>
<td>50 classifier genes and 5 control genes</td>
<td>70-gene profile</td>
<td></td>
</tr>
<tr>
<td>Technology</td>
<td>2 distinct epitope-specific monoclonal antibodies</td>
<td>Immunohistochemistry</td>
<td>Real-time RT-PCR</td>
<td>Quantitative RT-PCR</td>
<td>Microarrays</td>
</tr>
<tr>
<td>Laboratory</td>
<td>Centralized</td>
<td>Centralized</td>
<td>Centralized</td>
<td>Centralized</td>
<td>Centralized</td>
</tr>
<tr>
<td>Cost, $</td>
<td>~ 500–600</td>
<td>~ 4,000</td>
<td>Estimated to be ~ 2,000–3,000</td>
<td>~ 4,000</td>
<td></td>
</tr>
<tr>
<td>Clinical Use</td>
<td>Prognostic</td>
<td>Prognostic</td>
<td>Prognostic</td>
<td>Prognostic</td>
<td>Prognostic</td>
</tr>
<tr>
<td>FDA Approval?</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

FDA = US Food and Drug Administration, FFPE = formalin-fixed, paraffin-embedded, RT-PCR = reverse transcriptase–polymerase chain reaction.
ever, the assay does have limitations. For example, it is validated in hormone receptor–positive breast cancer alone. It also has a high false-negative rate for HER2 that could lead to the underestimation of recurrence risk because such results influence the recurrence score.\textsuperscript{55,66} In some cases, the recurrence score does not correlate with histological features of the tumor (ie, low-grade tumors with high recurrence scores). In such cases, inflammatory cells or a cellular stroma may artificially increase the recurrence score in low-grade invasive breast cancers.\textsuperscript{57} In addition to these issues, the strength of this genomic assay has been challenged by some researchers who suggest that similar results and prognostic information can be obtained using less costly and more widely available testing methods (eg, IHC).\textsuperscript{46,55} However, because Oncotype DX is performed in a centralized setting, problems with test reproducibility and issues of interpretation are minimized.\textsuperscript{68}

In an effort to predict the risk of recurrence of ductal carcinoma in situ (DCIS), the Oncotype DX DCIS score was introduced.\textsuperscript{69} Approximately 30\% of women with DCIS treated with breast-conservation surgery alone have local recurrence, and adjuvant radiotherapy has been shown to decrease the rate of recurrence by 50\% in such individuals.\textsuperscript{70} The Oncotype DX DCIS score is a 12-gene profile (7 cancer-related and 5 reference genes) assay that estimates individual 10-year risk of local recurrence (DCIS or invasive carcinoma) to determine whether adjuvant radiotherapy is likely to be beneficial in women with DCIS treated by local excision with or without tamoxifen. The test has been validated for\textsuperscript{69}:

- Low- to intermediate-grade DCIS with tumor size ≤ 2.5 cm
- High-grade DCIS with tumor size ≤ 1.0 cm with a minimum negative margin width ≥ 3 mm or no tumor on re-excision

The risk is stratified into low (< 39), intermediate (39–54), or high (≥ 55) categories.\textsuperscript{69} When used in this context, the Oncotype DX DCIS can predict which women might be spared from adjuvant radiotherapy. However, the score does not account for other important clinical and pathological features that may influence risk of recurrence, including tumor size, margin width, tumor grade, and necrosis. Therefore, if Oncotype DX DCIS is ordered outside the clinically validated variables, its results may be inaccurate and potentially risky to patients.\textsuperscript{71}

The Prediction Analysis of Microarrays (PAM50; NanoString Technologies, Seattle, Washington) was introduced in 2009 by Parker et al.\textsuperscript{72} It uses a quantitative RT-PCR assay on FFPE tissue, measuring the gene expression of 50 classifier and 5 control genes to identify luminal type A, luminal type B, HER2-enriched, and basal-like breast cancer.\textsuperscript{72} In addition to classifying subtypes of breast cancer, the test yields a risk of recurrence score, taking into consideration the pathological tumor size and a subset of quantitative values for proliferation, luminal gene expression, \textit{ESR1} (ER), \textit{PGR} (PR), and \textit{ERBB2}.\textsuperscript{52,55,72,73}

The PAM50 breast cancer intrinsic classifier test is recommended for patients diagnosed with invasive breast cancer, regardless of their stage or ER status.\textsuperscript{50,55,73,74} PAM50 has been shown in multivariate analyses to be an independent predictor of survival rate in breast cancer, as well as independent and superior to ER status, tumor grade, lymph node status, and other variables.\textsuperscript{50,55,73,74}

However, PAM50 does not entirely correlate with IHC results because the test may classify some tumors that are HER2-positive by standard techniques, such as IHC or in situ hybridization, as luminal types A or B. Conversely, up to 30\% of HER2-enriched tumors by PAM50 are clinically negative for HER2.\textsuperscript{52,75}

**Microarray-Based Multigene Assays**

The Symphony Genomic Breast Cancer Profile (Agenda, Irvine, California) is a comprehensive analysis of gene expression that includes MammaPrint (Agenda), BluePrint (Agenda), and TargetPrint (Agenda).

MammaPrint is approved by the US Food and Drug Administration to determine whether patients can safely avoid chemotherapy and its toxic adverse effects. BluePrint is a molecular subtyping profile that discriminates among 3 distinct molecular subtypes (luminal, basal-like, and HER2-enriched) and also guides treatment choices and combination therapies to optimize the treatment of breast cancer. TargetPrint tests for ER, PR, and HER2; therefore, it is used to determine whether patients with breast cancer are candidates for hormonal therapy or other targeted therapies.

For brevity, this article will focus its review on MammaPrint alone.

**MammaPrint**

MammaPrint is a microarray, in vitro, 70-gene expression profile that includes genes considered to be the hallmarks of local invasion, cancer-related biology, regulators of cell cycle, proliferation, metastasis, extravasation, survival in circulation, angiogenesis, and adaptation to the microenvironment.\textsuperscript{55} MammaPrint provides prognostic information, and its value is independent of conventional pathological and clinical factors (eg, HER2 status, tumor size, hormone receptor status).\textsuperscript{55} This test can also identify groups of patients at low risk within a node-positive population traditionally viewed as having a high risk for recurrence; thus, this assay has prognostic value in these individual patients.\textsuperscript{76} The test results are reported as low or high risk. Its prognostic use...
is approved for women under the age of 61 who have ER-positive or ER-negative, lymph node-negative breast cancer, and whose tumors are smaller than 5 cm in size. Those considered to be low risk per the results of the test should be advised to avoid adjuvant chemotherapy and instead receive endocrine therapy alone, whereas those at high risk are generally advised to receive chemotherapy. The test is highly accurate for patients at low risk because the likelihood of progression to metastatic disease in these cases is low. However, for patients at high risk, the prediction of metastatic progression is not as precise because only approximately 25% of these patients will progress at 5 years, which may be partly due to the use of adjuvant therapies in these patients. In addition, the majority of ER-negative patients will be classified as being high risk based on the results of the assay.

To determine whether individuals classified to be at low risk by MammaPrint but at high risk via the Adjuvant! Online tool could safely avoid chemotherapy, the Microarray In Node negative and 1-3 positive lymph node Disease may Avoid Chemotherapy (MINDACT) and MammaPrint trial with Adjuvant Online! was designed to randomize individuals with breast cancer and discordant results to receive either hormonal therapy alone or hormonal therapy in combination with chemotherapy. At the time of writing, the results of this trial were not available. The main limitation for the clinical use of MammaPrint is that it could not be performed on FFPE tissue, and tissue collected into an RNA preservative solution or fresh-frozen tumor samples was required. RNA retrieval from FFPE material is challenging due to the partial degradation of RNA during processing. Several investigators have tried to resolve this issue using archival FFPE tissue for clinical and research purposes, and their studies have demonstrated that applying DNA microarray analysis to FFPE tissue is possible, mainly due to improvements in technology and stringent protocols for tissue processing. A study by Sapino et al validated the MammaPrint assay on FFPE tissue blocks. The authors compared the results between fresh tissue samples and FFPE tissue in an independent series of matched tissue from 5 hospitals and found an overall equivalence rate of 91.5%, a precision rate of 97.3%, a repeatability rate of 97.8%, and highly reproducible results between replicate samples of the same tumor and between 2 laboratories (concordance rate, 96%). These results may open the door for the wide clinical use of MammaPrint.

Role of Pathologists in the Era of Molecular and Personalized Medicine

Because specialized molecular tests are aimed to deliver personalized cancer care, pathologists are presented with new and unique opportunities to directly engage in research and patient care. The practice of pathology has shifted from being purely diagnostic to having a central role on the medical team. Traditionally, pathology departments have acted as “safe keepers” or “custodians” of tissue samples removed from patients for treatment reasons, diagnostic reasons, or both. New challenges regarding the ethical, regulatory, and legal aspects involved in tissue management require pathologists to be informed about the technical aspects of appropriate specimen management and suitability.

Molecular testing is performed for diagnostic, clinical (to obtain predictive or prognostic information), or research purposes. The ethical, legal, and technical issues involved in each of these applications are, to some extent, different. Molecular tests developed for clinical uses are subject to stringent regulations and can only be performed in laboratories certified by the Clinical Laboratory Improvement Amendments (CLIA). Typically, these tests do not require informed consent unless they are performed to detect heritable genetic mutations such as BRCA1/2. Tests developed for research purposes alone do not need to undergo the same degree of federal and state regulations and can be performed in smaller, noncertified laboratories. Tests performed in laboratories not certified by CLIA cannot be formally reported or used for clinical management decisions.

The success of molecular medicine depends to a great extent on the quality of tissues to be tested. Preanalytic variables, such as tissue and biological sample collection, conservation, and transportation requirements, vary according to the type of test requested. Many molecular tests can be performed on FFPE tissue; few tests require fresh or frozen material. Fixation type, duration, and adequate tissue processing in the laboratory are crucial to ensure that genetic material is properly preserved. However, the role of the pathologist is not limited to supervision and guarantees of adequate tissue processing. Because of the broad spectrum of currently available molecular tests, no single laboratory is likely to be capable of offering them all. Pathology departments must have policies to establish how to handle “send-outs” for specialized tests, including clinical validity (medical necessity) of the test, adequate block and tissue selection, the release of tissue blocks or slides, covering the cost of supplies, shipping, and the test itself, among others.

The pathologist must also be aware of the amount of tissue needed for diagnostic purposes and make sure enough tissue remains to perform molecular studies, if needed, and when possible. The pathologist is also responsible for ensuring that enough tissue is available in the block to perform the requested test and to inform the testing facility about any deviation.
to specimen collection or processing (e.g., prolonged fixation time). In addition, the pathologist should select the tissue block with the greatest amount of invasive tumor, highest tumor grade (unless otherwise specified by the oncologist), and the least amount of in situ carcinoma, stromal inflammation, and biopsy changes. Testing results should also be correlated with histological findings to identify and resolve any discordant results.  

The pathologist also has a duty to ensure that, when material collected for diagnostic purposes, treatment purposes, or both types of purposes is then used for research, enough tissue remains for clinical tests that may be needed or developed in the future and that such research has been reviewed and approved by the corresponding Institutional Review Board.

Conclusions

Developments in molecular medicine help to identify biologically different subtypes of breast cancer and characterize the risk of recurrence as well as to predict treatment responses among patients with breast cancer. In addition to traditional prognostic and predictive factors, such as estrogen and progesterone receptors, several assays that support breast cancer prognostication in clinical practice are available. These tests have been designed to help oncologists and patients make appropriate decisions regarding the use of adjuvant systemic therapy in addition to surgery. However, molecular tests should be used together and not as a substitute to well-established pathological and clinical variables used in routine practice to evaluate patient prognosis (e.g., tumor grade, lymph node status). Technical advances in molecular medicine and the increasing number of molecularly tailored treatments have made it possible to potentially offer individualized treatment options to patients in the near future.

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