Breast cancer is a complex disease characterized by many morphological, clinical, and molecular features. For many years, breast cancer has been classified according to traditional parameters, such as histological type, grade, tumor size, lymph node involvement and vascular invasion, and biomarkers (e.g., estrogen receptor, progesterone receptor, and epidermal growth factor receptor 2), which are used in patient management. With emerging imaging techniques (i.e., digital mammography, tomosynthesis, ultrasonography, magnetic resonance imaging, nuclear medicine, and genomic techniques, such as real-time RT-PCR and microarrays), breast cancer diagnostics is going through a significant evolution. Imaging technologies have improved breast cancer diagnosis, survival, and treatment by early detection of primary or metastatic lesions, differentiating benign from malignant lesions and promoting intraoperative surgical guidance and postoperative specimen evaluation. Genomic and transcriptomic technologies make the analysis of gene expression signatures and mutation status possible so that tumors may be classified more accurately with respect to diagnosis and prognosis. The -omic era has also made possible the identification of new biomarkers involved in breast cancer development, survival, and invasion that can be gradually incorporated into clinical testing. These advances in both imaging and genomics contribute to more personalized and predictive patient management. We review the progress made in breast cancer diagnosis and management using these new tools. (Am J Pathol 2013, 183: 1075–1083; http://dx.doi.org/10.1016/j.ajpath.2013.07.002) For many years, breast cancer has been classified according to clinical and pathological criteria (i.e., histological features, grade, tumor size, lymph node involvement, and vascular invasion) (Figure 1). However, the predictive power of these criteria for selection of the optimal therapeutic approach is limited. In the past decade, significant advances in genomic and proteomic technologies have allowed researchers to better understand tumor cell biological characteristics and identify biological biomarkers involved in multiple signaling pathways that can improve general clinical practice [i.e., early detection, determination of clinical outcomes (prognosis), diagnosis, detection of recurrence after therapy, risk assessment, identification of targets for therapy, prediction of response to therapies (prediction), monitoring clinical outcomes of therapies, and imaging disease processes]. Rapid technological developments, such as real-time RT-PCR and microarrays, have allowed testing of numerous biomarkers and a more detailed classification of breast cancer, contributing to a personalized prognostic and predictive approach to management. Imaging techniques have also improved significantly during the past few years. According to Grizzle et al., imaging can detect primary or metastatic lesions and differentiate benign from malignant lesions. The necessity of novel biomedical imaging methods is to increase the frequency and accuracy with which disease can be detected and also to improve the prediction and monitoring of its progression or regression during treatment. Because significant technological progress has helped to individualize the diagnosis and treatment of breast cancer,
This review will provide an overview of the following: i) the latest developments in breast imaging and its importance for screening; ii) the markers clinically used for diagnosis, prognosis, and treatment; iii) the molecular subtypes of breast cancer; iv) the commercially available molecular signatures for predicting outcomes; and v) the importance of new biomarkers identified by array comparative genomic hybridization and next-generation sequencing.

**Screening/Diagnostics**

Imaging techniques (ie, digital mammography, tomosynthesis, ultrasonography, magnetic resonance imaging, and nuclear medicine) have improved breast cancer screening and show promise for intraoperative surgical guidance and postoperative specimen evaluation.

Mammography is considered the gold standard method of early breast cancer detection and diagnosis, but it has limitations, such as low sensitivity in dense breasts (Figure 2A). To improve mammographic sensitivity, complimentary imaging modalities, including digital breast tomosynthesis mammography (DBT) and contrast-enhanced digital mammography (CEDM), ultrasound, and magnetic resonance imaging (MRI), are often recommended. DBT is the basic mammography technique modified to capture multiple views of the breast at different angles and generate a three-dimensional (3D) image of the breast (Figure 2, C–F). According to Kilburn-Toppin and Barter, DBT has a high sensitivity and has been shown to improve lesion characterization, especially in women with dense breasts. DBT alone or in combination with CEDM results in a significant improvement in sensitivity. The CEDM technique uses the combination of an iodinated contrast agent with a mammography examination. It also has a high sensitivity and a better diagnostic accuracy than mammography alone and mammography with ultrasound.

Ultrasound imaging is used to detect, characterize, and guide biopsy of breast lesions (Figure 2B). For clinical examination, ultrasound screening is performed in combination with mammography. Because this technique also has some limitations (ie, inability to identify characteristics of benign and malignant lesions and inability to identify cancer in women with dense breasts), many modifications of this technique are being developed, such as 3D ultrasound, automated ultrasound, Doppler ultrasound, and sonoelastography.

Both mammography and ultrasound imaging alone are able to distinguish benign from malignant pathological conditions, with sensitivity values of 60.9% and 95.7%, and negative predictive values of 99.2% and 99.9%, respectively.

Recently, computer-aided detection or diagnostic (CAD) systems have been developed to help radiologists increase diagnostic accuracy, using either mammography or ultrasound. Computer-aided detection systems, CADe, were designed to assist radiologists in detecting and locating abnormal areas in images, and computer-aided diagnostic systems, CADx, were designed to diagnose and classify benign from malignant tissues. In mammography, these automated systems are classified into two groups: CADe, which generates a mammogram image with the abnormalities, and CADx, which helps the radiologist to grade them as benign or malignant. With ultrasound, CAD encompasses four stages: i) image processing, ii) image segmentation, iii) feature extraction and selection, and iv) classification.
MRI is effective for screening dense breasts, and by identifying additional occult lesions in the ipsilateral or contralateral breast, it can help determine whether lumpectomy or mastectomy (unilateral or bilateral) is the best treatment option. To locate the tumors, this technique requires the use of an intravenous contrast agent that highlights areas with dense vessel linkages, leading to many false positives. Thus, although MRI has a high sensitivity (94% to 100%), the specificity is low (37% to 97%). Lee et al suggested that the combination of MRI and mammography for screening might improve the chances of detecting early-stage cancers. However, MRI is not used routinely for screening.

Nuclear medicine has been used in oncological imaging for diagnosis, treatment decision, or treatment response monitoring. Positron emission tomography (PET) is a nuclear medicine screening technique that consists of the injection of radiolabeled ligands into the patient. PET produces 3D images based on the detection of altered physiological characteristics, rather than anatomical characteristics. Malignant cells frequently have increased glucose metabolism compared with normal cells. The radiolabeled ligands attach to glucose molecules, producing a contrast between cancerous and normal cells in PET images, providing information about the chemical functions inside organs and tissues. Computed tomography (CT) screening uses iodinated contrast intravenously to increase the contrast of the CT images to provide anatomical information. The PET-CT technique combines the advantage of PET, which indicates the metabolic activity of the malignant cells (based on glucose level), and CT, which identifies their location (Figure 2, G and H). The PET-CT procedure is able to distinguish benign from malignant breast tumors, and it also detects axillary metastases, with a sensitivity and specificity of 73% and 100%, respectively.

Although imaging techniques are improving the overall sensitivity and specificity of breast cancer screening and diagnosis.
diagnosis, they are mostly effective at indicating the location of the tumor within the breast. Some of them can often act as guides to disease extent, but the surgeon must still estimate the extent of disease, which may correlate with an increased risk of local recurrence and decreased survival. Therefore, techniques for real-time intraoperative surgical guidance and postoperative specimen evaluation are being developed and tested to improve the accuracy of surgical excision, reduce the recurrence rate, and increase the survival percentage.

Laughney et al\textsuperscript{12} developed a new spectroscopic imaging technique to detect and distinguish microscopic pathological features in resected breast tissues. This scatter spectroscopic imaging technique differentiates benign from malignant pathological conditions with 94\% accuracy in surgical tissues and identifies new imaging signatures, such as texture (correlation, contrast, and homogeneity) and shape (fractal dimension and Euler number) features to discriminate benign from malignant pathological conditions.

**Prognostic and Predictive Biomarkers**

Several biomarkers have been identified by high-throughput technologies, but only hormone receptors, such as estrogen (ER), progesterone (PR), and human epidermal growth factor receptor 2 (HER2), are routinely used clinically for breast cancer prognosis and therapeutic purposes (Figure 3).

**Estrogen Receptor**

Estrogen receptor, a member of the nuclear transcription receptor superfamily, is activated by steroid hormones, such as estrogen. Estrogen and its receptors are involved in several processes, including cellular proliferation, inhibition of apoptosis, invasion, and angiogenesis.\textsuperscript{13} ER has two isoforms, ER-\(\alpha\) and ER-\(\beta\), both expressed in the normal mammary gland; ER-\(\alpha\) is directly involved in pathological processes, including breast cancer. Estrogen receptor expression is considered one of the most important biomarkers in breast cancer and a positive target for anti-estrogen therapy.

Because estrogen inhibition can increase early breast cancer cure rates, improve the response rate in advanced disease, and reduce breast cancer incidence, ER-positive patients benefit from endocrine therapy, whereas ER-negative tumors do not. Tamoxifen is a selective modulator of ER that blocks steroid mechanisms preventing cellular replication and proliferation. The Early Breast Cancer Trials’ Collaborative Group\textsuperscript{14} reported that ER patients treated with tamoxifen had little effect on recurrence or breast cancer mortality in 5 years. Several studies have shown that 21\% to 33\% of ER-negative patients treated with neoadjuvant chemotherapy achieve a pathologically complete response, whereas only 7\% to 8\% of ER-positive patients achieved it.\textsuperscript{15,16}

Recently, some studies were performed to verify if there was differential benefit from aromatase inhibitors (AIs) and tamoxifen in different subgroups of breast cancer. Anastrozole, tamoxifen, alone or in combination (ATAC),\textsuperscript{17} and breast international group 1-98 (letrozole versus tamoxifen)\textsuperscript{18} trials showed that ER status did not lend a greater benefit from either AIs or tamoxifen.

**Progesterone Receptor**

The PR gene is an estrogen-regulated gene. Its expression depends on the presence of ER; thus, it is rarely seen in ER-negative tumors. Approximately half of the ER-positive tumors express PR.\textsuperscript{13} Although PR-negative patients have a worse outcome than PR-positive patients treated with tamoxifen, the relative benefit of this treatment is similar in both groups.\textsuperscript{17} In 2005, Dowsett et al\textsuperscript{19} (the ATAC trial on PR-negative and PR-positive patients) showed that the first group had greater benefit from anastrozole than tamoxifen. However, the breast international group 1-98 trial, performed by Viale et al,\textsuperscript{19} demonstrated that PR status did not affect outcomes with either AI or tamoxifen treatment.

**HER2**

HER2 belongs to the human epidermal growth factor receptor family of tyrosine kinases, which includes epidermal growth factor receptor (EGFR; HER1), HER3, and HER4. HER2 can regulate cell proliferation, survival, and other processes important for carcinogenesis. The activation of HER2, which occurs through gene amplification, leading to receptor protein overexpression, is identified in 15\% to 20\% of all breast cancers. This overexpression makes it a robust target for and predictor of the benefit from treatment with trastuzumab (Herceptin; Genetech, South San Francisco, CA). Trastuzumab is a monoclonal antibody that targets the extracellular domain of the HER2 receptor, blocking its activation.

Several technologies, such as immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), real-time RT-PCR, and enzyme-linked immunosorbent assay, are able to detect HER2 status, but only IHC and FISH have
been clinically used to detect HER2 gene amplification and/ or overexpression\textsuperscript{20} in a routine clinical setting.

IHC is a semiquantitative method that detects HER2 receptor expression on the cell surface using a grading system (0, absence; 1+, negative; 2+, equivocal; 3+, overexpression). Although this is the most routinely used method to detect HER2 status, it has some disadvantages (different fixation protocols, scoring systems, and antibody selection), which compromise its reproducibility and accuracy. FISH is a quantitative method that measures gene copy number. It is more reproducible and accurate when compared with IHC. To obtain more accurate results, the American Society of Clinical Oncology and the College of American Pathologists published a guideline with recommendations for HER2 evaluation.\textsuperscript{13}

**Molecular Profile of Breast Cancer**

Transcriptional profiling technologies (ie, gene expression microarrays) analyze the expression of thousands of genes simultaneously, allowing for a tumor molecular profile design. According to the literature, a quantitative study of multiple genes provides more accurate information about the molecular profile of the tumor than a conventional single marker.\textsuperscript{21,22}

Perou et al\textsuperscript{23} published the first article classifying breast cancer into intrinsic or molecular subtypes based on gene expression patterns using hierarchical clustering, which groups genes and samples according to similarity in their patterns of gene expression. This study classified breast cancer into four molecular subtypes: luminal, HER2 enriched, basal-like, and normal breast-like. In 2006, an expansion of this study in a larger group of patients showed that the luminal group could be divided into two categories, luminal A and luminal B.\textsuperscript{24} Furthermore, in 2007, the same group of researchers reported a new molecular subtype, claudin-low.\textsuperscript{25}

<table>
<thead>
<tr>
<th>Molecular subtype</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>HER2 positive</th>
<th>Basal-like (or TNBC)</th>
<th>Claudin-low (or TNBC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (%)</td>
<td>50-60 ER-related genes, ↓ proliferation genes</td>
<td>10-20 HER2-related genes, ↑ proliferation genes</td>
<td>15-20 Cks, P-cadherin, CAV1/2, CD44, KIT</td>
<td>10-20 HIGH ER, PR, HER2</td>
<td>12-14 HIGH ER, PR, HER2</td>
</tr>
<tr>
<td>Genetic expression profile</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Histological grade</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>IHC markers</td>
<td>ER\textsuperscript{+}, PR\textsuperscript{+}, HER2\textsuperscript{+}, Ki-67, CK8/18\textsuperscript{+}, GATA3\textsuperscript{+}</td>
<td>ER\textsuperscript{+}, PR\textsuperscript{+/-}, HER2\textsuperscript{+/-}, GATA\textsuperscript{+}, Ki-67</td>
<td>ER\textsuperscript{+}, PR\textsuperscript{-}, HER2\textsuperscript{+}</td>
<td>ER\textsuperscript{+}, PR\textsuperscript{-}, HER2\textsuperscript{+}, GATA3\textsuperscript{+}, CK5/6\textsuperscript{+}</td>
<td>ER\textsuperscript{+}, PR\textsuperscript{-}, HER2\textsuperscript{+}</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Good</td>
<td>Intermediate/poor to poor</td>
<td>Poor</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Response to chemotherapy</td>
<td>Low (7% CR)</td>
<td>Intermediate (17% CR)</td>
<td>High (43% CR)</td>
<td>High (36% CR)</td>
<td>Low</td>
</tr>
<tr>
<td>Treatment</td>
<td>AI and SERMs: tamoxifen</td>
<td>Tamoxifen and AI</td>
<td>HER2 target therapy</td>
<td>PARP-1 inhibitors</td>
<td>PARP-1 inhibitors</td>
</tr>
<tr>
<td>New treatment targets</td>
<td>PI3K/akt/mTOR pathway</td>
<td>PI3K/akt/mTOR pathway</td>
<td>PI3K/akt/mTOR pathway</td>
<td>PI3K/akt/mTOR and RAS-Raf-Mek pathway</td>
<td>PARP-1 inhibitors</td>
</tr>
</tbody>
</table>

CAV, caveolin; CK, cytokine; CR, complete response; GATA, GATA binding protein; KIT, v-KIT Hardy-Zuckerman 4 feline sarcoma viral oncogene human homolog; SERM, selective estrogen receptor modulator.

Recently, the mRNA expression subtypes, known as luminal A, luminal B, HER2, and basal-like, were analyzed using different platforms, including genomic DNA copy number arrays, DNA methylation, exome sequencing, mRNA arrays, miRNA sequencing, and reverse-phase protein arrays.\textsuperscript{26} This study evaluated individual platforms and integrated pathways identifying several subtype-specific mutations and copy number changes that might be therapeutically targetable. Table 1 summarizes the characteristics of the molecular subtypes.

**Luminal**

The luminal/ER\textsuperscript{+} breast cancers are the most heterogeneous in terms of gene expression, mutation spectrum, copy number changes, and patient outcomes.\textsuperscript{26} The main biological difference between luminal A and B subtypes is an increased expression of proliferation genes and an expression of EGFR and HER2 identified in luminal B tumors.\textsuperscript{22}

The luminal A subtype represents 50\% to 60\% of breast cancer cases. Its IHC profile is characterized by the expression of ER, PR, CK8/18, GATA3, and TP53, low expression of Ki-67, and absence of HER2 expression. This subtype is characterized by low histological grade and good prognosis. These tumors show a high frequency of PIK3CA mutation (49\%) and low TP53 mutation (12\%).\textsuperscript{26} The treatment of these patients is based on hormonal AIs in postmenopausal women and/or selective estrogen receptor modulators, such as tamoxifen.

The luminal B subtype represents 10\% to 20\% of all breast cancers. Compared with the luminal A subtype, they have a higher grade, worse prognosis, and worse proliferative index, leading to a more aggressive phenotype. These tumors are ER\textsuperscript{+}, PR\textsuperscript{+/-}, Her2\textsuperscript{+/-}, and EGFR\textsuperscript{+}, and have a high Ki-67. They also have a high frequency of PIK3CA (32\%) and TP53 (29\%) mutations, ATM loss, and MDM2 amplification.\textsuperscript{26} Although these patients have a worse
prognosis, 17% of them reach complete response when treated with neoadjuvant chemotherapy. They respond to either tamoxifen or AI.

Because luminal cancers have a high frequency of PIK3CA mutations, patients may be able to benefit from investigational drugs in human clinical trials that inhibit the phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway.

HER2 Enriched

This molecular subtype corresponds to 15% to 20% of all breast cancers. Among them, only 60% are clinically HER2+, and the rest are luminal B>B-luminal A>basal-like. It is characterized by overexpression of HER2- and HER2-associated genes, a high proliferative index, a high histological grade, a poor prognosis, and a high response to neoadjuvant chemotherapy (43% partial cytogenetic response). According to The Cancer Genome Atlas Network, HER2-positive tumors are divided into two subgroups: HER2 positive and luminal-HER2 positive. HER2-positive patients also show higher expression of RTK genes (FGFR4, EGFR, and HER2) and TP53, whereas luminal-HER2-positive patients show higher expression of the luminal cluster genes, such as GATA3 and ER. Approximately 30% to 40% of HER2 tumors are ER+. Of HER2-positive tumors, 75% have a TP53 mutation and 42% have a PIK3CA mutation. Patients are treated with trastuzumab, an anti-HER2 drug, but they can also benefit from the new therapeutic inhibitory molecules of the PI3K/AKT/mTOR pathway.

Basal-Like

The basal-like subtype represents 10% to 20% of breast cancer cases. They are characterized by a high proliferative index, a high histological grade, a poor prognosis, and a high response to neoadjuvant chemotherapy (36% partial cytogenetic response). Their IHC profile shows absence of ER, PR, and HER2 expression, and high expression of EGFR and CK5/6. Although they are clinically characterized as triple-negative breast cancer (TNBC), only 50% to 75% of them are basal-like. Basal-like tumors have a high frequency of TP53 mutations (84%), combined with loss of TP53 function and loss of RB1. They have been associated with germ-line mutations in BRCA1. According to Bosch et al, variations, such as mutations and epigenetic mechanisms, decrease BRCA1 gene function and predispose to the development of basal-like tumors, the absence of ER expression, and worse prognosis. Because most of the basal-like tumors are TNBC, it is difficult to identify the best therapeutic targets for these patients. Approximately 20% of patients have germ-line and/or somatic BRCA1 or BRCA2 variants, suggesting that they might benefit from poly-ADP ribose polymerase-1 (PARP1) inhibitors. The inhibition of PARP1 in patients with defective DNA repair by BRCA1 contributes to the accumulation of breaks in the double-stranded DNA and to cell death.

Several new targets, such as inhibitory molecules of the PI3K/AKT/mTOR and RAS-RAF-MEK pathway, might benefit this group. Eroles et al reported that basal-like cancers had amplified PIK3CA, KRAS, BRAF, and EGFR (49%, 32%, 30%, and 23%, respectively).

Claudin-Low

Claudin-low subtype tumors represent 12% to 14% of all breast cancers. They are characterized by low expression of cell-cell junction proteins, such as claudin-3, claudin-4, claudin-7, and E-cadherin. This subtype shows overexpression of genes involved in immune response, indicating an intense immune cell infiltration, and genes linked to mesenchymal differentiation and epithelial-mesenchymal transition. The IHC profile of this group shows absence of ER, PR, and HER2 expression, but approximately 20% of the tumors are positive for hormone receptors; therefore, they are not true TNBC. Claudin-low tumors have a high grade and a poor prognosis, because there is no specific treatment for these patients. According to Perou, patients with these tumors might benefit from PARP1 treatment, because they also have alterations in BRCA1 pathways.

Normal Breast-Like

Normal breast-like tumors correspond to 5% to 10% of all cases. They express genes present in the adipose tissue and lack expression of ER, PR, HER2, EGFR, and CK5. This tumor subtype has an intermediate prognosis and does not respond to neoadjuvant chemotherapy. Some researchers believe that normal breast-like tumor is a result of a technical artifact due to contamination of normal tissue.

Molecular Signature for Prediction

Recently, gene expression assays, such as MamaPrint (Agenda, Inc., Irvine, CA), Oncotype DX (Genomic Health, Redwood, City, CA), and the Genomic Grade Index (GGI; Affymetrix, Santa Clara, CA), have become commercially available. By using real-time PCR or microarray technology, these platforms identify a prognostic gene signature to predict response to therapy.

The MamaPrint test (approved by the US Food and Drug Administration) was developed from a gene expression profiling analysis known as the Amsterdam 70-gene profile or signature. This group of researchers identified a 70-gene prognostic signature using a microarray platform in node-negative breast cancer patients younger than 55 years. This signature consisted of genes involved in the cell cycle, invasion, metastasis, angiogenesis, and signal transduction. The 70-gene prognostic signature was validated in node-negative and node-positive tumors, as well as treated and untreated patients, and proved to be a robust predictor for distant metastatic-free survival, independent of adjuvant treatment, tumor size, histological grade, and age. A second
positive and lymph node—negative disease, treated with tamoxifen, have a 10-year distant recurrence chance.\textsuperscript{37} The 97-gene profile (GGI) was designed to grade tumors more accurately than the conventional histological grade, especially grade 2 tumors. Although the implementation of these molecular signatures may improve the clinical management of breast cancer patients, the cost of these tests is relatively high, approximately $4000 for MamaPrint and $3500 for Oncotype DX, when compared with the usual pathological testing, such as IHC.\textsuperscript{2}

A major issue in IHC is the accuracy for pre-analytic factors (ie, duration of fixation, type of processing, and type and intensity of the antigen used) and postanalytic factors (ie, slide scoring system) and the cutoffs used to determine positive and negative results. For this reason, typical pathological testing has been compared with these types of molecular signatures. Knauer et al\textsuperscript{38} identified that 80% of tumors classified as grade 1 by conventional methods were classified as low-risk prognosis by the MamaPrint test, while 20% were shown to be high risk by MamaPrint. For patients who were identified as grade 3, 88% were identified as high risk by the 70-gene profile and 12% showed low risk. Intermediate grade showed approximately 55% low and 45% high risk by MamaPrint.

**Future Directions**

Although transcriptomic analysis is a powerful tool to analyze multiple genes involved in breast cancer development and to identify new prognostic and prediction markers, the development of more sophisticated technologies, such as next-generation sequencing, epigenetics, proteomics, and metabolomics, is still needed for the following: i) to provide a better understanding of breast tumorigenesis, ii) to identify new driver genetic and epigenetic genes, iii) to characterize intratumor heterogeneity, iv) to identify mechanisms of resistance to therapy, and v) to identify new biomarkers for prognostics and prediction, resulting in better and more accurate monitoring for breast cancer.\textsuperscript{39}

Recently, miRNAs have been widely investigated because of their potential role as both novel biomarkers and targets for cancer therapy. It is known that miRNAs are involved in several biological processes, such as cell cycle regulation, cell growth, apoptosis, cell differentiation, and stress response.\textsuperscript{40} Tang et al\textsuperscript{41} observed a link between altered validation was performed in node-negative T1-2 breast tumors not treated with chemotherapy and compared with traditional clinical factors included in the Adjuvant! Online software (https://www.adjuvantonline.com/index.jsp, registration required, last accessed August 5, 2013). The 70-gene profile showed better prediction of time to distant metastasis and overall survival.\textsuperscript{13}

Oncotype DX, a 21-gene recurrence score (RS) prognostic indicator, predicts the probability of distant recurrence in node-negative patients treated with tamoxifen, and in those with ER\textsuperscript{+} breast cancer (Table 2).\textsuperscript{35} The assay identifies expression of 21 genes (5 reference genes and 16 genes associated with breast cancer), selected from a set of 250 genes previously studied by the National Surgical Adjuvant Breast and Bowel Project clinical studies. Real-time RT-PCR in formalin-fixed, paraffin-embedded samples was performed to quantify the expression of the 21 genes and calculate the RS, which classified patients into three groups: high, intermediate, and low risk. The 21-gene recurrence score was validated in 675 ER\textsuperscript{+}, node-negative, tamoxifen-treated patients from the National Surgical Adjuvant Breast and Bowel Project showing that RS correlated with distant recurrence, relapse-free interval, and overall survival, independent of age and tumor size. Oncotype DX testing also includes markers used routinely in diagnoses, such as ER, PR, and HER2.

The GGI signature was developed to reclassify patients with histological grade 2 tumors, which is informative for clinical decision making. Sotiriou et al\textsuperscript{36} analyzed microarray data from 189 invasive breast cancers and identified 97 genes associated with histological grade, most of them involved in cell cycle regulation and proliferation (Table 2). These genes were differentially expressed between high- and low-grade breast tumors. The intermediate-grade tumors showed an expression pattern similar to either high- or low-grade cases. The GGI may increase the accuracy of tumor grading and improve treatment decisions.

Although MamaPrint is applicable to either hormone receptor—positive or hormone receptor—negative and lymph node—positive or lymph node—negative patients, Oncotype DX and GGI tests have a limited scope. The 21-gene profile (Oncotype DX) was designed to predict distant recurrence during a 10-year period and to predict the response to chemotherapy of lymph node—negative and hormone-positive tumor. Approximately 15% of patients with ER-
miRNA and breast cancer development and metastasis, either through the loss of tumor-suppressor miRNAs or the overexpression of oncogenic miRNAs in breast cancer cells.

Conclusions

Advances in imaging methods for breast cancer screening have increased the frequency and accuracy with which breast cancer is detected. They have also improved the prediction and monitoring of disease during treatment. Rapid developments, using new molecular technologies, have provided new information about the biological characteristics of the tumor and led to a reclassification of breast cancer from a molecular standpoint. These genomic techniques reveal new biomarkers involved in neoplastic development, survival, and invasion that can be gradually incorporated into clinical testing. Together, these advances, in both imaging and genomics, have contributed to a more personalized management of the breast cancer patient.

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