Molecular Testing and the Pathologist’s Role in Clinical Trials of Breast Cancer

Hyo Sook Han,1 Anthony M. Magliocco2

Abstract

Molecular characterization of breast cancer is pivotal for identifying new molecular targets and determining the appropriate treatment choices. Advances in molecular profiling technology have given greater insight into this heterogeneous disease, over and above hormone receptor and human epidermal growth factor receptor 2 status. Agents targeting recently characterized molecular biomarkers are under clinical development; the success of these targeted agents is likely to depend on identifying the patient population most likely to benefit. Therefore, clinical trials of breast cancer often require prescreening for, or stratification by, relevant molecular markers or exploratory analyses of biomarkers that can predict or monitor the response to treatment. Consequently, the role of the pathologist has become increasingly important. The key considerations for pathologists include tissue availability, ownership of archival tissue, type of diagnostic/biomarker test required, method of sample processing, concordance between different tests and testing centers, and tumor heterogeneity. In the present review, we explore how pathology is used in current clinical trials of breast cancer and describe the various technologies available for molecular testing. Furthermore, the factors required for the successful application of pathology in clinical trials of breast cancer and the issues that can arise and how these can be circumvented are discussed.

Introduction

Breast cancer constitutes one of the most common types of cancer, affecting approximately 232,670 women and 2360 men in the United States in 2014.1 It is also the second most common cause of cancer death in women.1 The heterogeneous nature of breast cancer, in both clinical pathologic and histologic examination, has long since represented a challenge to identifying the appropriate treatment for patients with this disease.2 Traditionally, breast cancer tumors were classified according to the histologic features and slide-based techniques that categorized breast tumors as ductal or lobular carcinoma and determined the nodal status, size, and grade of the tumor.3,4 During the past decade or so, with advances in molecular genotyping technologies, several molecular subtypes of breast cancer have been identified, dependent on hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status, that differ in prognosis and chemotherapy responsiveness (Table 1). The vast majority of breast cancers are epithelial carcinomas and therefore are the focus of the present review. Broadly speaking, the main intrinsic subtypes of epithelial breast cancer include luminal A, luminal B, HER2+, and basal-like cancer.2,5-8,13-16 Further subtypes based on androgen receptor (AR) status have also been proposed.13

The identification of specific molecular subtypes of breast cancer has paved the way for a more personalized approach to the treatment of breast cancer using targeted therapies. Moreover, the clinical benefit observed with agents targeting hormone signaling or HER2 has confirmed the importance of identifying further molecular targets. Advances in molecular profiling have recently unveiled several genetic and epigenetic alterations that are likely drivers of breast tumor biology.7 Some of these genetic alterations can help distinguish the current molecular subtypes of breast cancer (Table 1), and others can form their own molecular class, such as phosphatidylinositol 3-kinase (PI3K) pathway aberrations, which are present at high frequencies across all current molecular subtypes.7

Additional molecularly targeted agents for breast cancer are now in clinical development. The success of these new, targeted therapeutic agents will depend on identifying the patient populations most likely to respond and on characterizing biomarkers that can
monitor the response to treatment. As such, the role of the pathologist in clinical trials of breast cancer is changing from a primarily histologic/cytologic diagnostic role to a critical role in analyzing molecular biomarkers and the changing molecular profile of the cancer during treatment. In addition, the need for sufficient material for molecular profiling and for repeated analyses during the course of treatment has expanded the types of tissue examined by the pathologist, and with it, the pathologist’s repertoire of tools and techniques.

The present review provides an overview of how pathology is being used in breast cancer trials, including the available and novel techniques used in molecular testing of breast cancer. We also discuss the key technical and legal considerations required to execute well-designed clinical trials that can improve our understanding of the etiology and treatment of breast cancer.

### Molecular Testing in Breast Cancer

Molecular testing in breast cancer can be used to diagnose tumor types, recognize hereditary implications (e.g., *BRCA1* mutations), identify appropriate therapeutic agents (e.g., HER2+ disease), determine the prognosis of the disease, and identify biomarkers that can predict or monitor the response to treatment.17,18 The major techniques used in molecular testing in breast cancer trials are summarized in Table 2.

#### Immunohistochemistry and Cytogenetics

HER2 is a clear prognostic marker and therapeutic target for breast cancer, and accurate assessment of HER2 status is critical for the optimal treatment of patients.18 The techniques for diagnosing HER2+ breast tumors are primarily based on immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH), both of which can be automated and allow for concurrent assessment of tumor morphology, minimizing the appearance of artefacts.18,32

### Table 1 Molecular Subtypes of Breast Cancer Tumors

<table>
<thead>
<tr>
<th>Molecular Feature</th>
<th>HR+ (ER+ and/or PR+)</th>
<th>HER2+</th>
<th>TNBC (HR−, HER2−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical intrinsic subtype</td>
<td>Luminal A (HER2−/−)</td>
<td>Luminal B (HER2+/−)</td>
<td>HER2</td>
</tr>
<tr>
<td>Frequency among breast cancers</td>
<td>40%-60%</td>
<td>~15%</td>
<td>~10%</td>
</tr>
<tr>
<td>Genetic profile</td>
<td>Low expression of proliferative genes (e.g., Ki-67); high expression of <em>ESR1</em>, <em>GATA3</em>, <em>FOXA1</em>, <em>KRP1</em>, and <em>MYB</em>, frequent mutation of <em>MAP3K1</em> and <em>MAP2K4</em></td>
<td>Lower expression of ER-related genes; increased expression of proliferation genes; frequent amplification of <em>CCND1</em> (cyclin D); fusion of <em>ESR1</em> and <em>CCDC170</em> genes</td>
<td>High expression of HER2 and related genes; increased expression of proliferation genes; high genomic instability; frequent <em>TP53</em> mutations</td>
</tr>
<tr>
<td>Grade</td>
<td>Lower (moderately to well differentiated)</td>
<td>Higher (poorly to moderately differentiated)</td>
<td>High (poorly differentiated)</td>
</tr>
<tr>
<td>Prognosis</td>
<td>Intermediate</td>
<td>Intermediate</td>
<td>Generally poor</td>
</tr>
<tr>
<td>Targeted therapy</td>
<td>Endocrine (tamoxifen, letrozole, anastrozole, exemestane, fulvestrant)</td>
<td>Endocrine (tamoxifen, letrozole, anastrozole, exemestane, fulvestrant)</td>
<td>Anti-HER2 (trastuzumab, pertuzumab, T-DM1, lapatinib)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>Lower response</td>
<td>Intermediate response</td>
<td>Responds to anthracycline-based chemotherapy</td>
</tr>
</tbody>
</table>

**Abbreviations:** *BRCA1* = breast cancer 1, early onset; *CCDC170* = coiled-coil domain containing 170; ER+ = estrogen receptor-positive; *ESR1* = estrogen receptor 1; *HER2−/−* = human epidermal growth factor receptor 2-positive/negative; HR+/− = hormone receptor-positive/negative; *MAP2K4* = mitogen-activated protein kinase 4; *MAP3K1* = mitogen-activated protein kinase 1; *PARP* = poly ADP ribose polymerase; PR+ = progesterone receptor-positive; T-DM1 = trastuzumab emtansine; TNBC = triple-negative breast cancer; *TP53* = tumor protein 53.
Molecular Testing and Pathologists’ Role

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Molecular Assays Used in Breast Cancer Clinical Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IHC</strong>&lt;sup&gt;19-21&lt;/sup&gt;</td>
<td><strong>FISH</strong>&lt;sup&gt;20&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Description</strong></td>
<td>Use of antibodies to detect levels of a specific protein</td>
</tr>
<tr>
<td><strong>Detection</strong></td>
<td>Protein expression levels</td>
</tr>
<tr>
<td><strong>Example assays</strong></td>
<td>HER2 FISH pharmDX Kit, PathWysion HER2 DNA Probe Kit&lt;sup&gt;28&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Sample requirement</strong></td>
<td>Tissue sections; typically cut from FFPE samples; phosphorylated epitopes might instead require snap freezing and alcohol fixation</td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>Simple, inexpensive procedure; processed slides can be stored for years and reasessed; cell morphology can be viewed in parallel</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Issues with reproducibility; semiquantitative, subjective score; fixation time can affect results; results dependent on quality of antibody used to detect the protein; usually only 1-2 proteins can be analyzed per section</td>
</tr>
</tbody>
</table>

Abbreviations: cDNA = complementary DNA; DNA-seq = DNA-sequencing; FFPE = formalin-fixed paraffin-embedded; FISH = fluorescent in situ hybridization; HER2 = human epidermal growth factor receptor 2; IHC = immunohistochemistry; NGS = next-generation sequencing; PAM50 = prediction analysis of microarray 50; PCR = polymerase chain reaction; RNA-seq = RNA-sequencing; RT = reverse transcription; SNP = single nucleotide polymorphism.

In particular, in the diagnosis of HR<sup>+</sup> and/or HER2<sup>+</sup> tumors.<sup>56</sup> Examples of computerized image analysis platforms include the Aperio ScanScope (Aperio, Vista, CA) and the Definiens (Munich, Germany) Tissue Studio. The Aperio ScanScope system allows the scanning of whole glass slides to create a digital image that can be analyzed using specific algorithms within a short period.<sup>36</sup> The Definiens Tissue Studio system detects regions of interest and distinguishes cells and subcellular attributes within the target regions (e.g., cell membrane for HER2 analysis or nucleus for HR analysis).<sup>36</sup> In addition, tissue microarrays are a high-throughput method that allows multiple analyses to be performed for multiple patients on a single microscope slide.<sup>34,40</sup> Up to approximately 1000 tissue samples can be arrayed into a single paraffin block and IHC or FISH performed simultaneously on multiple samples and under identical conditions.<sup>34,40</sup> The high-precision tissue microarray instrument (typically Beecher Instruments, Sun Prairie, WI) acquires tissue cores from predefined regions of donor tissue sample paraffin blocks and places them at precise coordinates on an empty recipient block.<sup>40</sup> Small sections (5 μm) can be cut from the tissue microarray blocks to generate tissue microarray slides for molecular and IHC analyses. This technology enables simultaneous analysis of multiple targets at the DNA, RNA, and protein levels on a large number of specimens. It also allows for experimental uniformity and helps
conserved valuable archival tissue samples.\(^{40}\) Taken together, such advances can improve workflow efficiency and yield more accurate results, leading to an increased role for the pathologist in diagnostics and personalized medicine.

**Genomic Techniques**

Several different techniques are available to assay a group of genes at once. Oncotype DX (Genomic Health Inc.) is a quantitative reverse transcription-polymerase chain reaction (RT-PCR) assay that measures the expression of 21 genes (16 cancer-related genes and 5 reference genes that serve as internal controls).\(^{41}\) This assay was developed to provide a Recurrence Score in women with tamoxifen-treated, lymph node-negative, ER\(^{+}\) early-stage breast cancer and has been optimized for formalin-fixed paraffin-embedded tumor samples.\(^{41}\) The 21 genes are derived from a set of 250 genes likely to be prognostic for HR\(^{+}\) breast cancer.\(^{37}\) The Kaplan-Meier estimates of the rates of distant recurrence at 10 years in patients with low (<17), intermediate (18-30), and high (≥31) Recurrence Scores were shown to be 7%, 14%, and 31%, respectively.\(^{41}\) Women with a high-risk score have been shown to benefit from adjuvant chemotherapy, but those with a low-risk score derive minimal benefit from chemotherapy.\(^{42}\) However, although HER2, ER, and PR genes are part of the 21-gene set,\(^{41}\) the HER2 and HR status should be independently assessed.\(^{33}\) To date, Oncotype DX is the only multigene breast cancer assay recommended by the main breast cancer management guidelines for its use as a prognostic test and its ability to predict the likelihood of adjuvant chemotherapy benefit in node-negative, ER\(^{+}\), HER2\(^{-}\) early-stage breast cancer.\(^{44-47}\) Although the Oncotype DX assay has become the most widely used clinical gene expression assay in the United States, similar prognostic information was found to be provided by the IHC4 score,\(^{27}\) which is a simpler and less expensive alternative.\(^{37}\)

MammaPrint (Agenda) is a DNA microarray-based 70-gene assay, which involves hybridization of complementary DNA to specific probes to determine the gene expression levels.\(^{48}\) The test was developed using breast cancer specimens from women who had undergone surgery but had received no systemic therapy for primary breast cancer and had been followed up for ≥5 years.\(^{48}\) The 70 genes were selected from a microarray of 25,000 genes.\(^{48}\) Using the MammaPrint assay, the patients can be divided into low- or high-risk groups, corresponding to a 10-year distant metastasis-free survival rate of >90% or <90%, respectively.\(^{37}\) The TRANSBIG validation study showed that patients classified as low risk using the MammaPrint assay had a 10% chance of distant recurrence and those classified as high risk a 29% chance of recurrence, without adjuvant treatment at 10 years.\(^{49,50}\) Several studies have provided support for the predictive power of MammaPrint for the response to adjuvant or neoadjuvant chemotherapy.\(^{37}\) MammaPrint has a broader indication than Oncotype DX by including both ER\(^{+}\) and ER\(^{-}\) patients and has been approved by the FDA for use as a prognostic test for women with lymph node-negative breast cancers.\(^{37,53}\) Unlike Oncotype DX, however, MammaPrint is not currently approved as a predictive test of chemotherapy benefit. Further research is required to validate the predictive capabilities of this test.

Using microarray and RT-PCR data, a 50-gene prediction analysis of microarray (PAM50) assay was developed to provide a standardized method of breast cancer classification into the intrinsic subtypes (luminal A, luminal B, HER2\(^{+}\), and basal-like), together with a continuous risk of recurrence score (based on the gene signature, intrinsic subtype, tumor size, and proliferation score).\(^{51}\) A recent study found that the incorporation of selected proliferation and pathologic features that generate the risk of recurrence score with the PAM50 assay provided more prognostic information than the Oncotype DX Recurrence Score in patients with endocrine-treated, ER\(^{+}\), and node-negative disease, with fewer patients categorized as intermediate risk and more as high risk.\(^{52}\) PAM50 can also identify which HER2\(^{+}\) breast cancers are most likely to benefit from trastuzumab-based chemotherapy.\(^{53}\) This originally quantitative RT-PCR assay has now been developed by NanoString Technologies into Prosigna, a DNA hybridization-based assay that uses the PAM50 gene signature for the diagnosis of the intrinsic subtype of breast tumors from FFPE tissue samples.\(^{30}\) Prosigna has recently been approved by the FDA and European, Australian, and Canadian marketing authorities as a prognostic indicator for distant recurrence-free survival at 10 years in postmenopausal women with HR\(^{+}\) breast cancer who have undergone surgery in conjunction with locoregional treatment consistent with the standard of care.\(^{54-56}\) However, it is important to note that additional criteria for the Prosigna indication vary between countries. The FDA-approved indication for Prosigna in the United States is lymph node-negative stage I or II or lymph node-positive stage II breast cancer.\(^{54}\) In Europe, Australia, and Canada, Prosigna has been approved for use in lymph node-negative stage I or II or lymph node-positive stage II or IIIA breast cancer to be treated with adjuvant endocrine therapy alone, when used in conjunction with other clinicopathologic factors.\(^{55,56}\)

The IMPAKT 2012 working group evaluated the validity of 6 different prognostic genomic tests, including the Oncotype DX, MammaPrint, PAM50, Genomic Grade Index (Ipsogen), Breast Cancer Index (Biotheranostics), and EndoPredict (Sividon Diagnostics).\(^{29}\) Using available data, they found Oncotype DX and MammaPrint to have convincing analytical validity and convincing clinical validity in patients with ER\(^{+}\) breast cancer.\(^{29}\) The other tests showed a clear association with prognosis, but the IMPAKT 2012 working group concluded that further data, including from larger population sizes, was required for convincing clinical validity.\(^{29}\) None of the tests provided convincing clinical utility. The IMPAKT 2012 working group reported that models need to be developed that integrate the clinicopathologic factors and genomic tests.\(^{29}\) The European Society for Medical Oncology Clinical Practice Guidelines for the diagnosis, treatment, and follow-up of early breast cancer suggest that gene expression profile tests, including MammaPrint, Oncotype DX, Prosigna, and EndoPredict, might be used to help make treatment decisions in conjunction with all clinicopathologic factors, when decisions might be challenging or in cases of uncertainty regarding indications for adjuvant chemotherapy.\(^{37}\)

**Next-Generation Sequencing Technologies**

Next-generation sequencing (NGS) can sequence an entire genome (all DNA) or exome (coding gene regions) in 1 run to profile mutations, rearrangements, amplifications, or deletions (DNA sequencing). NGS can also be used to analyze the entire transcriptome (cellular RNA, RNA sequencing) to determine gene expression levels, splicing patterns, and RNA editing as well as some
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gene rearrangements and mutations. Such a broad approach enables the discovery of new genetic abnormalities that can predispose to, or characterize, a particular tumor type.

NGS technologies afford an unprecedented sequencing depth (ie, the number of times a single base within the genome is sequenced), enabling rare sequences to be detected.59,60 For instance, a tumor that contains a large amount of normal tissue would require greater sequencing depth to detect the tumor mutations. This is also important for overcoming issues of tumor heterogeneity, in which a particular clone might be especially underrepresented.

Slightly more targeted approaches can also be used in which large panels (a few hundred) of cancer-specific genes are selectively sequenced 59-61. These NGS panels require less total sequencing and afford in-depth coverage for the genes of interest.31 A range of NGS panels featuring different gene sets are available, including those from companies such as Foundation Medicine, Qiagen, Life Technologies, and Illumina, as well as independent laboratories and academic institutions.31 A recent study found that the use of an NGS panel for patients with solid tumors, the most frequent being breast cancer (26% of patients), identified potentially actionable alterations in most patients, additional therapeutic options and facilitated enrollment into clinical trials.62 NGS panels are also frequently used in exploratory analyses of genetic biomarkers in clinical trials.

NGS-based assays that can detect gene mutations from small amounts of DNA are also in development; for example, to analyze tumor DNA from fine needle aspirates or circulating tumor DNA (ctDNA) from blood samples.63-66 Several studies across various tumor types have shown a high degree of concordance between mutations in ctDNA (detected using NGS techniques) and mutations from the primary tumors.63,64,66 In a study of 17 patients with metastatic breast cancer, the Ion AmpliSeq Cancer Hotspot Panel, version 2 (Ion Torrent) was used to analyze 69 tumor samples (primary and metastases) and 31 plasma samples.67 Tumor and plasma results were concordant in 76% of patients; complementary information was provided by discordant results (24% of patients).67 A separate study of 6 patients with advanced breast, ovarian, and lung cancer who were followed up for >1 to 2 years demonstrated that exome-wide analysis of ctDNA could identify the development of resistant mutations to certain therapies, including mutations in PIK3CA (after paclitaxel treatment), RB1 (after cisplatin treatment), and EGFR (T790M; after gefitinib treatment).68 The GUARDANT360 assay, a single-molecule next-generation digital sequencing assay that detects mutations in ctDNA across a panel of 54 genes, has also demonstrated promising results using samples from patients with breast cancer.69,70

The introduction of NGS and other high-throughput genome-wide technologies into the clinical setting is set to significantly alter breast cancer diagnosis and management. However, certain obstacles must be overcome before such technologies are incorporated into routine clinical practice. The SAFIR01 trial, which tested the ability of comparative genome hybridization arrays and Sanger sequencing to guide therapeutic decisions in patients with metastatic breast cancer, demonstrated limited clinical benefit (9% objective response and 21% stable disease) for inclusion of these techniques in decision-making.71 NGS technologies generate a vast amount of data, which require specialized storage, processing, and analysis programs that might not be easily implemented in small diagnostic laboratories.72 Distinguishing between mutations that drive tumor growth and development and other “passenger” mutations also represents a challenge to clinical practice. Finally, ethical and legal concerns regarding the protection of patients’ genetic information need to be resolved before genome-wide technologies can be used in the clinical setting.72

Biomarkers in Clinical Trials

The association between drug responsiveness and the presence of, or change in, biomarkers is increasingly being tested across different tumor types in clinical trials. This includes proof-of-concept trials, which only enroll patients with specific biomarkers (integral biomarkers); trials in which enrollment is stratified according to presence or absence of biomarkers; and trials that prospectively investigate the effect of biomarkers on drug response from tissue samples acquired during enrollment (integrated biomarkers).73

The incorporation of biomarkers into clinical trials aims to allow more accurate identification of patients who are likely to benefit from certain therapies and to provide a more comprehensive view of how novel therapies work. However, introducing biomarkers into clinical trials remains somewhat challenging, particularly owing to the need for standardization of assays across multiple countries and clinical practices. HER2 testing in clinical trials has been controversial owing to the potential discordance between local and central laboratories and between assay methods (IHC vs. FISH). For example, a small subgroup of patients who were included in the adjuvant National Surgical Adjuvant Breast and Bowel Project (NSABP)-B31 trial on the basis of HER2 status on central laboratory analysis were confirmed to have HER2 status on central laboratory analysis.74 Despite central HER2-negativity, these patients showed a significant benefit from trastuzumab, suggesting that adjuvant trastuzumab might benefit patients regardless of HER2 amplification status.74 The IHC assay results can be influenced by variations in sample preparation, choice of antibody, and subjective assessment of the staining intensity.75 Careful assay design and validation and the collection of appropriate specimens are therefore required to generate meaningful results from the inclusion of biomarker analyses in clinical trials. Tissue availability also poses a challenge for incorporation of biomarkers into clinical trials. However, if such challenges can be overcome, trials of this nature have the potential to provide great value in terms of personalized medicine.

Examples of biomarker-driven clinical trials in breast cancer are highlighted in Tables 3 and 4. I-SPY 2, a randomized trial with an adaptive study design, is underway in the neoadjuvant setting. I-SPY 2 is investigating several different experimental treatment regimens in women with newly diagnosed, locally advanced breast cancer at high risk of disease recurrence.80 Patients are randomly assigned to standard neoadjuvant chemotherapy or neoadjuvant therapy plus an experimental regimen according to the patient’s tumor biomarkers.82 Standard biomarkers (including intrinsic subtype classification and the MammaPrint assay) are used to determine patient eligibility and randomization; predictive and exploratory biomarkers will be evaluated during the trial.81 The adaptive trial design enables early data from the trial to be used to guide treatment decisions for patients later in the trial and minimize the exposure of patients to treatments that will not benefit them.80 In the metastatic setting,
<table>
<thead>
<tr>
<th>Trial Name/ID</th>
<th>Agent(s)</th>
<th>Phase</th>
<th>Patient Population</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PI3K/AKT/mTOR Pathway Inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SANDPIPER; NCT02340221</td>
<td>Fulvestrant ± taselisib (GDC-0032; Genentech/Roche; beta-sparing PI3K inhibitor)</td>
<td>III</td>
<td>Postmenopausal women with unresectable, locally advanced or metastatic ER⁺, HER2⁻, PIK3CA-mutant breast cancer refractory to an aromatase inhibitor; n = 600</td>
<td>Ongoing</td>
</tr>
<tr>
<td>SOLAR-1; NCT02437318</td>
<td>Fulvestrant ± alpelisib (BYL719; Novartis; PI3k inhibitor)</td>
<td>III</td>
<td>Men and postmenopausal women with HR⁺, HER2⁻ advanced breast cancer with identified PIK3CA status and progression on or after aromatase inhibitor treatment; n = 820</td>
<td>Ongoing</td>
</tr>
<tr>
<td>BELLE-2; NCT01610284</td>
<td>Fulvestrant ± buparlisib (BKM120; Novartis Pharmaceuticals; pan-PI3K inhibitor)</td>
<td>III</td>
<td>Postmenopausal patients with locally advanced or metastatic HR⁺, HER2⁻ breast cancer refractory to an aromatase inhibitor; stratified by PI3K pathway activation status; n = 1149</td>
<td>No longer recruiting</td>
</tr>
<tr>
<td>NCT01923168</td>
<td>Letrozole ± buparlisib (BKM120; Novartis Pharmaceuticals; pan-PI3K inhibitor) or alpelisib (BYL719; Novartis Pharmaceuticals; PI3k inhibitor)</td>
<td>II</td>
<td>Postmenopausal women with HR⁺, HER2⁻ primary breast cancer; 2 cohorts: PIK3CA mutant and PIK3CA wild-type; n = 360</td>
<td>Ongoing</td>
</tr>
<tr>
<td>BEECH; NCT01625286</td>
<td>Paclitaxel ± AZD5363 (AstraZeneca; AKT inhibitor)</td>
<td>I/II</td>
<td>Advanced or metastatic breast cancer (ER⁺, HER2⁻ in phase II part); stratified by PIK3CA mutation status in phase II part; n = 140</td>
<td>Ongoing</td>
</tr>
<tr>
<td>FAKTION; NCT01992952</td>
<td>Fulvestrant ± AZD5363 (AstraZeneca; AKT inhibitor)</td>
<td>I/II</td>
<td>Postmenopausal women with advanced ER⁺, aromatase inhibitor-resistant breast cancer; mutualional status of PIK3CA and loss of PTEN assessed at baseline; n = 150</td>
<td>Ongoing</td>
</tr>
<tr>
<td>NCT02457910</td>
<td>Talazoparib (BMN 673; BioMarin Pharmaceutical; PARP inhibitor) vs. physician’s choice</td>
<td>III</td>
<td>Locally advanced and/or metastatic breast cancer with deleterious or pathogenic germline BRCA1 or BRCA2 mutation; n = 270</td>
<td>Ongoing</td>
</tr>
<tr>
<td>ICEBERG 1; NCT00494234</td>
<td>Olaparib (AZD2281, KU0059436; AstraZeneca/KuDOS Pharmaceuticals; PARP inhibitor)</td>
<td>II</td>
<td>Advanced breast cancer after failure on previous chemotherapy; BRCA1 or BRCA2 mutation; n = 81</td>
<td>No longer recruiting</td>
</tr>
</tbody>
</table>
the phase II SAFIR02 trial is using NGS of metastatic lesions from patients with HER2+ breast cancer to randomly assign patients to receive the appropriate targeted therapy versus standard therapy.76 This follows from the SAFIR01 study that suggested the feasibility of personalization of medicine for metastatic breast cancer.71

In the HR− breast cancer arena, several biomarker-driven or stratified clinical trials are being conducted to assess the efficacy of both well-established and pipeline PI3K pathway inhibitors in specific patient populations and to elucidate mechanisms of resistance (Table 3). The phase III BELLE-2 study is investigating buparlisib (BKM120), a pan-PI3K inhibitor, combined with fulvestrant in postmenopausal women with locally advanced or metastatic breast cancer that is hormone receptor-negative and/or PTEN-negative. In studies of neoadjuvant everolimus therapy.83 The results suggested that tumors with PIK3CA exon-9 mutations, high Ki-67 (a marker for proliferation), HER2-positivity, or high levels of phosphorylated AKT, might benefit from neoadjuvant everolimus therapy.

In HER2+ breast cancer, the effect of biomarkers on treatment outcome was investigated as part of the phase III CLEOPATRA (clinical evaluation of pertuzumab and trastuzumab) study, which demonstrated improved efficacy of the anti-HER2 monoclonal antibody, pertuzumab, combined with trastuzumab and docetaxel versus placebo plus trastuzumab and docetaxel in the first-line treatment of patients with HER2+ metastatic breast cancer.87 Prospective biomarker analysis of CLEOPATRA revealed that elevated HER2 protein, high HER2 and HER3 mRNA levels, wild-type PIK3CA, and low serum HER2 extracellular domain showed a significantly better prognosis, with PIK3CA showing the greatest prognostic effect.87 Therapy targeting ARs is also being studied in HER2+ breast cancer; therefore, some clinical trials are testing for AR status. For example, a phase II trial of enzalutamide (an AR antagonist) combined with trastuzumab is being investigated in locally advanced or metastatic breast cancer that is HER2+, hormone receptor-negative, and ER/PR-negative.83

Molecular biomarkers are also being used for patient selection in trials of triple-negative breast cancer (TNBC; ER−, PR−, and HER2−). TNBC is a heterogeneous disease, and a recent study identified 6 distinct TNBC subtypes.88 A proportion of TNBCs are characterized by defective BRCA-dependent homologous recombination. These tumors appear to be responsive to agents that lead to DNA double-strand breaks, such as platinum salts and poly (ADP-ribose) polymerase inhibitors.89 Identification of tumors with BRCA-dependent homologous recombination classically involved identifying germline mutations in the BRCA1 or BRCA2 genes. Recently, gene signatures that suggest inactivation of homologous recombination in breast tumors in the absence of BRCA1 or BRCA2 germline mutations, termed BRCAness, have been developed.89 In a recent clinical trial, patients with tumors classified as BRCA-like by array comparative genomic hybridization were found to have a

### Table 3

<table>
<thead>
<tr>
<th>Trial Name/ID</th>
<th>Agent(s)</th>
<th>Phase</th>
<th>Patient Population</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT0188923976</td>
<td>Enzalutamide (MDV3100; Medivation; AR antagonist)</td>
<td>II</td>
<td>AR+ TNBC; n = 118</td>
<td>No longer recruiting</td>
</tr>
<tr>
<td>NCT0209196076</td>
<td>Enzalutamide (MDV3100; Medivation; AR antagonist) + trastuzumab</td>
<td>II</td>
<td>Locally advanced or metastatic AR+, HER2+, and ER/PR− breast cancer; n = 80</td>
<td>Ongoing</td>
</tr>
<tr>
<td>NCT0199020976</td>
<td>Orteronel (TAK-700; Takeda/Millennium Pharmaceuticals; androgen biosynthesis inhibitor)</td>
<td>II</td>
<td>AR+, metastatic breast cancer; n = 86</td>
<td>Ongoing</td>
</tr>
<tr>
<td>NCT0236869176</td>
<td>Enobosarm (GTx-024; GTx, Inc.; SARM)</td>
<td>II</td>
<td>AR+ TNBC; n = 55</td>
<td>Ongoing</td>
</tr>
<tr>
<td>NCT0235398876</td>
<td>Bicalutamide (AstraZeneca Ltd.; nonsteroidal antiandrogen) vs. physician’s choice</td>
<td>II</td>
<td>AR+ TNBC; n = 60</td>
<td>Planned</td>
</tr>
</tbody>
</table>

**Abbreviations:** AR = androgen receptor; BRCA = breast cancer susceptibility gene; ER+/- = estrogen receptor-positive/negative; HER2+/- = human epidermal growth factor receptor 2-positive/negative; HR+/- = hormone receptor-positive/negative; IC = immunohistochemistry; mTOR = mammalian target of rapamycin; PARP = poly (ADP ribose) polymerase; PIK3CA = phosphatidylinositol 3-kinase catalytic subunit alpha; PI3K = phosphatidylinositol 3-kinase; PTEN = phosphatase and tensin homolog; SARM = selective androgen receptor modulator; TNBC = triple-negative breast cancer.
<table>
<thead>
<tr>
<th>Trial Name/ID</th>
<th>Agent(s)</th>
<th>Phase</th>
<th>Patient Population</th>
<th>Status</th>
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<tr>
<td>NCT01528345</td>
<td>Dovitinib (TKI-258; Novartis Pharmaceuticals; FGFR, VEGFR, and PDGFR inhibitor) plus fulvestrant</td>
<td>II</td>
<td>Postmenopausal patients with locally advanced or metastatic HR⁺, HER2⁻ breast cancer; progression during or after endocrine treatment; stratified by FGF amplification and visceral disease; n = 97</td>
<td>Completed</td>
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<tr>
<td>NCT01484041</td>
<td>Dovitinib (TKI-258; Novartis Pharmaceuticals; FGFR, VEGFR, and PDGFR inhibitor) plus an aromatase inhibitor</td>
<td>I/II</td>
<td>HR⁺; HER2⁻ breast cancer (primary or metastatic) resistant to an aromatase inhibitor; tumor must be available for central testing for FGFR1 amplification; n = 36</td>
<td>No longer recruiting</td>
</tr>
<tr>
<td>NCT02202746</td>
<td>Lucitanib (Clovis Oncology; FGFR, VEGFR, and PDGFR inhibitor)</td>
<td>II</td>
<td>Relapsed or refractory metastatic breast cancer; different cohorts based on FGFR1 or 11q amplification; n = 201</td>
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<td>FINESSE; NCT02053636</td>
<td>Lucitanib (Clovis Oncology; FGFR, VEGFR, and PDGFR inhibitor)</td>
<td>II</td>
<td>Metastatic ER⁺ breast cancer after hormonal therapy; different cohorts based on FGFR1 or 11q amplification; n = 123</td>
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<td>RADICAL; NCT01791985</td>
<td>AZD4547 (AstraZeneca; FGFR inhibitor) plus letrozole or anastrozole vs. exemsorine</td>
<td>I/II</td>
<td>ER⁺ breast cancer (primary or metastatic); progression on anastrozole or letrozole; mandatory tumor biopsy for confirmation of FGFR status by FISH for phase IIa; n = 56</td>
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<td>PANACEA; NCT02129556</td>
<td>Pembrolizumab (MK-3475; Merck; PD-1 monoclonal antibody) plus trastuzumab</td>
<td>II/II</td>
<td>Advanced, trastuzumab-resistant, HER2⁺ breast cancer; presence of PD-L1 expression as assessed by IHC; n = 46</td>
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<tr>
<td>METRIC; NCT01997333</td>
<td>Glematurumab vedotin (CDX-011; Celldex Therapeutics; anti-gpNMB antibody) vs. capcetabine</td>
<td>II</td>
<td>Metastatic gpNMB-overexpressing TNBC; n = 300</td>
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<tr>
<td>NCT01828021</td>
<td>Margetuximab (MGAH22; MacroGenics; anti-HER2 antibody)</td>
<td>II</td>
<td>Relapsed or refractory advanced breast cancer that is HER2⁺ by IHC with no HER2 gene amplification by FISH; n = 41</td>
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<tr>
<td>NCT00673829</td>
<td>Anti-CEA designer T cells ± interleukin-2</td>
<td>I</td>
<td>Metastatic or unsectectable locally advanced breast cancer with CEA expression; n = 26</td>
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<td>NCT02349724</td>
<td>Anti-CEA designer T cells</td>
<td>I</td>
<td>Relapsed or refractory CEA⁺ lung, pancreatic, gastric, breast, or colorectal cancer; n = 75</td>
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<td>NCT01837602</td>
<td>cMet CAR RNA T Cells</td>
<td>I</td>
<td>Metastatic breast cancer refractory to ≥1 standard therapy or newly diagnosed operable TNBC, both with positive cMET expression; n = 15</td>
<td>Ongoing</td>
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</tbody>
</table>

Abbreviations: CAR = chimeric antigen receptor; CEA = carcinoembryonic antigen; CTA = cancer tests antigen; ER = estrogen receptor; FGFR = fibroblast growth factor receptor; FISH = fluorescence in situ hybridization; gpNMB = glycoprotein NMB; HER2⁺/⁻ = human epidermal growth factor receptor 2-positive/negative; HR⁺ = hormone receptor-positive; IHC = immunohistochemistry; PD-1 = programmed cell death protein 1; PDGFR = platelet-derived growth factor receptor; PD-L1 = programmed cell death ligand 1; poly-ICLC = polyinosine/cytosine stabilized with poly-L-lysine and carbocymethylcellulose; TKI = tyrosine kinase inhibitor; TNBC = triple-negative breast cancer; VEGFR = vascular endothelial growth factor receptor.
Role of the Pathologist

Our enhanced understanding of the molecular concepts underlying the etiology of breast cancer and the advent of targeted therapeutics and personalized medicine has meant that the role of pathologists in breast cancer trials has changed considerably. During the past decade, the role of the pathologist has shifted from that of tumor diagnosis and histologic classification toward more functional pathology, including the identification of biomarkers of therapeutic response and new targets for therapy.

Pathologists are now involved in various stages of clinical trials, from pretrial/eligibility screening through to the determination of response to therapy. In clinical trials of breast cancer, pathologists are required for determining the molecular profile of a tumor. In addition to classifying the tumor into one of the main subtypes according to the HR and/or HER2 status, trials of targeted therapy often require testing for other specific molecular markers (eg, markers of PI3K pathway activation, BRCA1/2, or mutations in growth factor receptors). These molecular markers can be prerequisites for enrollment or can be used for patient stratification before randomization. An equally important role for pathologists lies in quality control and ensuring the correct technologies are applied. To this end, centralized testing of HER2 is typically used in clinical trials of anti-HER2 agents to ensure consistency across centers.

Pathologists also have a critical role in assessing the primary endpoints. The pathologic complete response (pCR), defined as the absence of residual disease after surgery, is a common endpoint in neoadjuvant trials and is assessed by the pathologist. CAP has provided guidelines to assist pathologists in reporting the examination of breast specimens at surgery. However, the exact definitions of pCR can vary considerably and little, if any, agreement has been reached regarding the precise definition of this endpoint. In a study of >6000 patients from 7 prospectively conducted clinical trials of neoadjuvant anthracycline and taxane-based chemotherapy, the pCR definition of no residual invasive or in situ cancer within both the breast and the lymph nodes has been shown to be associated with longer disease-free and overall survival than the absence of the breast alone or the presence of residual ductal carcinoma in situ (DCIS).

In contrast, another study reported that residual DCIS did not adversely affect the outcomes and therefore did not appear to be prognostic. The investigators of the latter study argued that residual DCIS should be included in the definition of a pCR.

Pathologists also play a critical role in determining the effect of biomarkers or molecular alterations on response to therapy and in monitoring the markers of response to determine the effectiveness of therapy. Different tissues, including peripheral blood mononuclear cells, platelet-rich plasma, skin, and hair, are under investigation as surrogates for tumor tissue and can be used by pathologists to assess the pharmacodynamic effects of anticancer agents in clinical trials. Such tissues are readily available, easily accessible, and can be sampled repeatedly with limited invasiveness. In addition, circulating tumor cells (CTCs) can be isolated from blood and potentially serve as a “liquid biopsy.” Studies have demonstrated that a change in the number of CTCs can be an early marker of response to therapy, and their molecular characterization can be representative of the current tumor molecular status. However, the predictive ability of CTC biomarker analysis has demonstrated variable and inconclusive results. Reports from the HER2+ metastatic setting have indicated that CTC counts can lose their prognostic significance after treatment with HER2-directed therapy. Furthermore, anti-HER2 or anti-epidermal growth factor receptor (EGFR) treatment in patients with HER2+ or EGFR+ CTCs (who had HER2− primary tumors) has been associated with limited or no response. Additional studies are therefore necessary before CTC analyses can be introduced into clinical practice. Tumor cells also release nucleic acids into the bloodstream, and ctDNA might reflect the molecular characteristics of the primary tumor. The use of ctDNA has demonstrated significant sensitivity in monitoring tumor progression and early tumor responsiveness to therapy in metastatic breast cancer.

Overall, pathologists have an essential and varied role in all stages of breast cancer clinical trials. Pathologists can therefore provide important input into clinical trial designs and should be involved in the process as early as possible. Pathologists can advise on the type of tissue collected, the techniques used, and the definition of
endpoints, all of which can depend on the type of trial and sample availability. Failure to include the pathologist in clinical trial protocol development can result in less suitable investigations and analyses, a potential need to retest tumor samples, and, ultimately, a delay in patients receiving treatment.

Considerations for Pathologists

The availability of adequate tissue samples represents a major challenge in clinical trials, particularly in the retrospective setting, where access and retrieval of tissue samples and the degradation of specimens over time can hinder the analyses. Moreover, significant legal and ethical considerations exist relating to ownership of the archival tissue material that must be considered. Although it is clear how tissue samples should be used in clinical trials using integral biomarker testing (eg, determining HER2 status for patient enrollment), the procedures and regulations involved in the retrieval and use of samples for post-trial biomarker analyses are more complex. Clarity is lacking regarding who owns the specimens and for what purposes the specimens can be used, and informed consent for further use of tissue samples is often required from patients. In addition, considerable disparity exists among the hospitals and governing authorities in how these specimens can be managed.

For some institutions, the decision remains with the patient, and for others, it lies with the laboratory; this presents a unique challenge in multicenter clinical trials. In the event of competing trials requiring the same tissue specimens, the lack of clear guidance also generates an ethical challenge for the laboratory when trying to prioritize how and for which trials the tissue specimens can be used. All these considerations can lead to delays in the analysis of archival tumor samples and, thus, delays in clinical trial enrollment and treatment administration.

It should also be determined whether repeat diagnostic processes will be performed on tissue received by the pathologist. Pathologists might automatically perform their own diagnosis if they are unaware that a diagnosis had been previously established or if they believe they are liable for providing their own diagnosis if they have not had the opportunity to review the previous results. Such repeat diagnostic processes can leave limited material for molecular testing. Moreover, pathologists might wish to perform their own testing regardless of the previous results owing to the critical nature of accurate pathology reporting. Confirmation of a diagnosis or biologic marker through repeat assessment of tissue is essential to ensure correct and appropriate treatment decisions are being made. In addition, an error in the pathology report could lead to the incorrect entry of a patient into a clinical trial. Should a discrepancy in the diagnoses be found, this can also have malpractice implications, and the legal implications surrounding these issues need careful consideration.

Prospective studies might allow the collection of tissues according to the protocols; however, proper diagnostic processing might limit sample availability for exploratory analyses. Furthermore, effective communication between clinicians and pathologists is critical for determining the type of sample to be collected, the method for sample processing, and the downstream tests that are required, which all depend on the nature of the clinical trial. It is also important to consider whether the samples will be processed “in house” or will be sent externally for the management of the tissue samples and handling steps. Furthermore, a wide array of technical challenges exists for tissue preservation and sample preparation. Fixation procedures can cause damage to the genomic DNA, and variability in these procedures is likely to affect the results. Although formalin-based fixation is thought to be the reference standard, delays in formalin fixation have been shown to impede HR and HER2 analysis, and brief fixation followed by rapid processing has proved effective for ER IHC in core biopsy specimens. The ASCO/CAP guidelines have recommended that any specimen used for HER2 testing should undergo the fixation process as soon as possible within 1 hour and fixed in 10% neutral buffered formalin for 6 to 72 hours. The time to fixation and the time in the fixative should also be recorded. Other factors that affect tissue preservation include intraoperative ischemia and hypoxia, previous biopsy, type of fixative used, and method of paraffin block preparation. Although the use of fresh or frozen tissue samples can overcome many of these issues and generally yield high-quality DNA, these techniques are costly and not always feasible. The optimization and standardization of these procedures will help improve the quality of tissue available for clinical research. In addition, developing a tissue bank, in which the remaining tumor tissue that has been removed during surgery or biopsy can be stored, could prove beneficial and ensure the availability of samples for future testing.

Other factors to consider are the rates of concordance among different techniques, specimens, and testing centers. In the ALTTO (Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation) trial, different assays demonstrated variability in the detection of HER2 and ER status that was evident even at the central testing centers. Discordance between assays can have detrimental effects on treatment and clinical trial enrollment, resulting in failure to treat a patient who might benefit from targeted therapy or the unnecessary treatment (with the associated costs and safety implications) of a patient. A comparison of local and central testing of HER2 status in a substudy of the VIRGO trial revealed discordance between testing centers in the identification of HER2 positivity in the study. This supports the ASCO/CAP recommendations to retest HER2 cases in certain situations, coupled with close communication between pathologists and clinicians. The HER2 gene is located in the long arm of chromosome 17 and polysomy of chromosome 17 can also affect HER2 testing. Polyomy of chromosome 17 can result in discordant HER2 testing by ISH, depending on whether the mean HER2 copy number or the HER2/CEP17 ratio is used. In addition, the regulation and effect of HER2 expression could differ between cases of selective HER2 amplification and an increase in copy number of part or all of chromosome 17. Tumor purity is another important consideration for molecular testing. Low tumor purity from admixture with stromal, vascular, or inflammatory cells can dilute the relevant biomarkers and promote false-negative results by decreasing assay sensitivity.

Different regulations for diagnostic testing could also be in place, and, in Europe, local regulations for diagnostic testing can differ between countries. In addition to the US FDA, another regulatory body governing diagnostic tests in the United States is the Clinical Laboratory Improvement Amendments program. Clinical Laboratory Improvement Amendments regulates laboratory developed
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tests (LDTs) in the United States, which are tests designed, manufactured, and used within a single laboratory.121,122 However, compared with the FDA scheme, the analytical validity of the Clinical Laboratory Improvement Amendments program is limited to the specifications of the particular laboratory where the test is performed and does not address the clinical validity or the relevance of the test.121 Although the FDA currently regulates only commercially marketed laboratory tests, the increasing complexity and widespread use of LDTs has led to the FDA proposing to regulate LDTs under guidelines similar to those for commercially marketed tests.122,123 This proposal is still under discussion among the authorities, industry, and academia.123

A further important consideration is tumor heterogeneity, which can lead to false-negative results and, thus, treatment failure and drug resistance.117,124,125 The high proliferation rate of tumor cells, combined with genomic instability and increased mutation rates, a common feature of tumor cells, promotes substantial genetic diversification of tumor cell populations.125 This can result in intratumor heterogeneity, such that a single tumor biopsy specimen might not be representative of the entire tumor.125 Furthermore, intratumor heterogeneity and Darwinian selection of driver mutations that potentially confer a growth or survival advantage can facilitate clonal succession and lead to treatment failure.124,125 This also means that archival tumor samples might not reflect the current molecular status of the tumor. Similarly, the accumulation of molecular alterations over time, known as tumor evolution/adaptation, presents a challenge when relying on archival tumor samples.125,126 Emerging techniques for analyses of CTCs or ctDNA could provide a less invasive approach to sampling the present tumor molecular profile.103,110 Metastasis of the tumor to other sites also often involves molecular changes that transform tumor cells into an aggressive form.127 Therefore, tumor heterogeneity can exist between primary and secondary or metastatic tumors. A significant proportion of regional or distant breast cancer relapse cases have been shown to exhibit changes in either ER or HER2 status from the primary tumor, which would have a dramatic impact on therapeutic strategy.128 The ASCO/CAP guidelines have recommended evaluation of HER2 status in all patients with newly diagnosed breast cancer and on metastases, in a metastatic site, if a tissue sample is available.33

Overall, pathologists must consider multiple variables such as tumor purity and heterogeneity and need to keep abreast of new technologies and updated procedures. Therefore, continuing medical education is particularly important, and pathologists should receive regular updates on this rapidly changing field. Furthermore, it is essential that all pathologists are properly educated regarding each clinical trial they are involved in, including the purpose, protocol, types of analyses required, and the questions under investigation. In return, pathologists must make every endeavor to guarantee the accuracy of molecular-based tests by ensuring appropriate specimen collection and tissue handling before an assay is performed, by the timely communication of results, and/or by reviewing and correlating the results with the clinicians; all of which are reliant on good communication between the pathologist and clinician.

Optimizing Pathology Practices

The field of molecular testing in breast cancer is fast expanding, with the addition of new technologies and procedures for sample preparation and analysis. The development of comprehensive guidelines and implementation of routine training practices for the collection and processing of tissue samples is therefore critical, and laboratory tests are now coming under closer scrutiny by the FDA. Different trials often have different requirements for the preparation and analysis of tissue samples; thus, the specimen types and analytic methods for each trial should be independently assessed and carefully considered.

Developing processes that accelerate and prioritize molecular testing will play a key role in improving the outputs of clinical trials. To this end, a fundamental need exists to improve communication between the oncologist and pathologist and to increase coordination between teams. It is pertinent that pathologists are provided with sufficient information about the requested tumor samples to be able to perform the procedures using the most effective method. The use of pathology in clinical trials can be further optimized if the pathologist is involved early on in the development of the trial protocol, including developing the study design and determining patient eligibility, patient stratification, quality control, treatment evaluation, and the use of biomarkers.129 Issues, such as tissue banking and prospective or retrospective biomarker development, also need to be addressed during clinical trial design.129

Conclusion

In this new era of molecular testing and personalized medicine, the role of the pathologist in clinical trials of breast cancer is changing. Recent advances in molecular analyses of breast tumors have revealed differential molecular properties that affect the prognosis, responsiveness, and resistance to therapy. The importance of molecularly profiling breast tumors has meant that a multitude of high-throughput assays and techniques are now entering clinical practice. These techniques have shown promise in enhancing the quantity and quality of information that can be obtained from clinical trials and might overcome some of the challenges of traditional assays. To improve the outcomes for patients with breast cancer, clinical trials must increasingly be designed to involve the use of biomarkers in patient selection, as surrogate endpoints for clinical response, to identify suitable patient populations for therapy, and/or to determine the mechanisms of resistance. Thus, the role of the pathologist in clinical trials has become complex and varied. In addition to diagnosis, the pathologist must manage complex testing and information, provide stewardship to the tissues, coordinate data from multiple laboratories, and generate comprehensive reports. It is clear that the pathologist should be involved in multiple stages during clinical trial development, including study design and data analysis. The lack of consultation with the pathologist can lead to delays or errors in sample processing and ultimately delays in patients receiving treatment. With the continuing evolution of molecular technologies, the pathologist is set to play a pivotal role in both drug discovery and the clinical treatment of patients with breast cancer.

Acknowledgments

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Disclosure

H.S. Han declares no competing interests; A.M. Magliocco has served as an advisor to Genoptix, Ventana Medical Systems, Bristol-Myers Squibb, DiaCarta, Guardant Health, BioTheranostics, and Janssen, has been an invited speaker for Leica Biosystems and Illumina, and has attended advisory board meetings for Novartis. None of these relationships have shaped the content of our report.

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