Breast Cancer Pathology in the Era of Genomics

Hannah Y. Wen, MD, PhD[a], Laura C. Collins, MD[b,*]

KEYWORDS

• Breast cancer • Germline testing • Special histologic subtype • Multigene assay • Ki67 • PD-L1 • Mutation profiling • ctDNA

KEY POINTS

- Genomic medicine offers the potential to identify the molecular underpinnings of a patient's breast cancer guiding targeted therapeutic options.
- In early-stage breast cancer, histopathology and tumor biomarker information, supported by multigene assays, for women with ER-positive disease, are fundamental in guiding treatment.
- Genomics in breast pathology is utilized for risk stratification, tumor classification, predictive/prognostic testing, identification of actionable targets, and monitoring for disease progression or treatment resistance.

INTRODUCTION

The era of genomic medicine provides an opportunity for pathologists to offer greater detail about the molecular underpinnings of a patient's cancer and thereby more targeted therapeutic options. For patients with breast cancer, the principal application at this time is in the advanced stage or metastatic setting. In early-stage breast cancer, routine histopathology along with breast tumor biomarker information (estrogen receptor [ER], progesterone receptor [PR], and human epidermal growth factor receptor 2 [HER2]), supported by multigene assays, for women with ER-positive breast cancers, remain fundamental in guiding treatment decisions.

In this review article, the role of genomics in breast cancer pathology, as it pertains to risk management, classification of special tumor types, predictive and prognostic testing, identification of actionable therapeutic targets, and monitoring for disease progression or development of treatment resistance is discussed.

SOURCE OF FUNDING SUPPORT: H. Wen is supported in part by a National Institutes of Health/National Cancer Institute Cancer Center Support Grant (P30CA008748).

[a] Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA; [b] Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

* Corresponding author.

E-mail addresses: weny@mskcc.org (H.Y.W.); lcollins@bidmc.harvard.edu (L.C.C.)

Hematol Oncol Clin N Am 37 (2023) 33–50
https://doi.org/10.1016/j.hoc.2022.08.004 **hemonc.theclinics.com**

GERMLINE TESTING

About 5% to 10% of breast cancers are hereditary, with *BRCA1* and *BRCA2* germline mutations accounting for most such cases.[1] Other breast cancer susceptibility genes include *PALB2, CHEK2, ATM, CDH1, PTEN, TP53*, and *STK11*, these being associated with lower lifetime risks for breast cancer than *BRCA1* and *BRCA2*.

The advent of multigene panel testing has enabled comprehensive detection of pathologic mutations, which can inform risk-reducing strategies, such as enhanced screening, prophylactic surgery, and chemoprevention. In addition, with the emergence of new treatment options, such as polyadenosine diphosphate-ribose polymerase (PARP) inhibitors, *BRCA* germline testing is not only a strategy for surveillance and prevention but also has become a predictive marker for PARP inhibitor treatment.[2–6] Furthermore, as next-generation sequencing technology is increasingly used in germline analysis, detection of germline variants beyond *BRCA1/2* is readily accomplished. Studies have shown pathogenic or likely pathogenic germline variants in 17% of patients with advanced cancer, including therapeutically actionable germline alterations in 8% of patients.[7,8] Thus, in patients with advanced cancer, germline sequencing analysis could have a complementary role to tumor sequencing analysis for therapy selection.

TUMOR CLASSIFICATION

In patients with newly diagnosed breast carcinoma, accurate categorization of tumor type, grade, and biomarker status, along with tumor size, the presence or absence of lymphovascular invasion, and axillary lymph node metastases, guide management. Most breast carcinomas are invasive carcinomas of no special type (NST, also known as invasive ductal carcinoma). Approximately 10% to 15% of breast carcinomas are invasive lobular carcinomas, with the remaining 5% together comprising the special histologic subtypes, such as tubular, mucinous, invasive cribriform, and invasive micropapillary carcinomas, among others. Almost 70% of breast carcinomas are ER positive; 15% to 20% demonstrate HER2 overexpression/amplification and 10% are ER, PR, and HER2 negative (triple negative). Genomic testing is not necessary for the diagnosis and management of these more frequently occurring carcinomas, and molecular subtyping with assays such as the PAM50 to provide the intrinsic tumor subtype (luminal A or B, HER2 enriched, or basal) are not indicated in routine clinical practice. Broadly speaking, ER-positive, HER2-negative tumors, or luminal-like carcinomas, have a more indolent clinical course than HER2-positive or triple-negative carcinomas (which overlap with basal-like).

As will be discussed in a later section, multigene assays, in conjunction with clinical and pathologic features, are used to guide the need for adjuvant chemotherapy in women with early-stage ER-positive breast cancer; patients with HER2-positive disease or triple-negative carcinomas (NST) receive adjuvant or neoadjuvant chemotherapy regimens. There are, however, some special subtypes of ER-, PR-, and HER2-negative breast cancers that have a more indolent clinical course, and for those patients, chemotherapy is not indicated. It is this small subset of triple-negative tumors for which genomic assays may be helpful to ensure accurate tumor classification so as to avoid overtreatment with chemotherapy. Increasingly, antibodies are being made available to some of the fusion proteins resulting from gene rearrangements present in various cancers auguring in the advent of molecular immunohistochemistry as a more readily accessible and affordable diagnostic tool.[9]

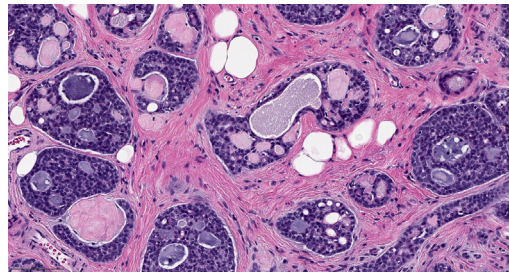

Fig. 1. Adenoid cystic carcinoma. In this conventional adenoid cystic carcinoma, the tumor is readily recognized by its cribriform growth pattern, the presence of a mixed population of epithelial and myoepithelial cells, and the deposition of basement membrane material in "pseudolumens."

ADENOID CYSTIC CARCINOMA

Adenoid cystic carcinoma is an uncommon breast cancer type comprising less than 1% of all breast carcinomas. In most cases, the histologic pattern of this tumor is readily recognizable, being composed of both epithelial and myoepithelial cells arrayed in a cribriform or trabecular pattern with the production of basement membrane material contained within pseudolumens created by the myoepithelial cells (Fig. 1). In addition to these morphologically characteristic patterns, there is a solid, basaloid variant that bears greater resemblance to conventional high-grade triple-negative breast cancer and that may be difficult to distinguish on microscopic examination alone (Fig. 2).[10–14]

The molecular alteration characteristic of adenoid cystic carcinoma is translocation and fusion of *MYB* or *MYBL1* with either *NFIB* or other gene partners.[15,16] The resulting gene fusion can be identified through cytogenetic analysis using a dual break-apart probe to MYB or through sequencing analysis. An immunohistochemical assay using an MYB antibody is also available, but although sensitive, this is less specific (see Fig. 2).[17,18]

Although most adenoid cystic carcinomas are considered low grade and have an indolent clinical course, the solid basaloid variant often demonstrates high nuclear grade and may have zones of necrosis prompting concern for more aggressive behavior.[12,14] There are insufficient data on the outcome of this particular variant of adenoid cystic carcinoma to inform a specific management recommendation, but

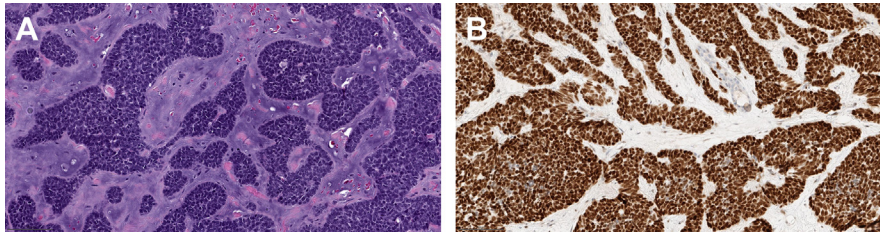

Fig. 2. Solid basaloid variant of adenoid cystic carcinoma. (*A*) This variant of adenoid cystic carcinoma can be more challenging to recognize, given the solid growth pattern, the often higher grade nuclei and the relative absence of obvious basement membrane material (hematoxylin and eosin stain). (*B*) MYB immunostain. Nuclear expression of MYB can be helpful in supporting the diagnosis.

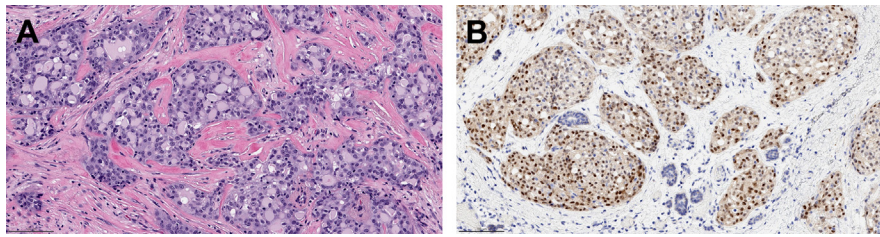

Fig. 3. Secretory carcinoma. (*A*) Secretory carcinoma can be a mimic for other types of breast carcinoma. Here the tumor displays a microcystic or cribriform type growth pattern, with luminal secretions. The tumor cells are relatively bland. (*B*) pan-TRK immunostain. Nuclear positivity is supportive of the diagnosis of secretory carcinoma.

accurate diagnostic categorization will help build knowledge on the prognosis of this tumor for future treatment decisions.

SECRETORY CARCINOMA

Secretory carcinoma is another uncommon triple-negative (or low ER-positive) tumor subtype with indolent behavior. It has a particular predisposition for occurring in children, although a broad age range of individuals may be affected. There are characteristic morphologic features that should raise the pathologic differential diagnostic consideration of secretory carcinoma, such as cells with finely vacuolated cytoplasm, the presence of secretions in the ductular lumens, and bland tumor cell nuclei (**Fig. 3**); however, the architectural growth pattern can vary considerably (circumscribed, solid, microcystic, infiltrative) and the occasional presence of a central scar or sclerotic area can confound. Again, in adult women, the diagnosis of a triple negative breast cancer without the qualifier of this special histologic subtype may result in overtreatment.

An *ETV6::NTRK3* gene fusion characterizes secretory carcinoma.[19] As with the *MYB::NFIB* gene fusion in adenoid cystic carcinoma, this gene fusion can be identified through cytogenetic analysis with a dual break-apart probe to ETV6 or NTRK3. A pan-TRK immunohistochemical antibody is available and can be used to screen for this tumor subtype (see **Fig. 3**).[20,21] This, in conjunction with confirmation of the *ETV6::NTRK3* gene fusion either with in situ hybridization or sequencing analysis, can be used to support diagnosis and treatment decisions.

Most patients with secretory carcinoma are managed with surgical excision alone. Rare cases of recurrence and late metastases have been reported.[22] Such patients have been demonstrated to benefit from treatment with pan-TRK inhibitors[23] emphasizing the potential role for molecular analysis in therapeutic decision-making.

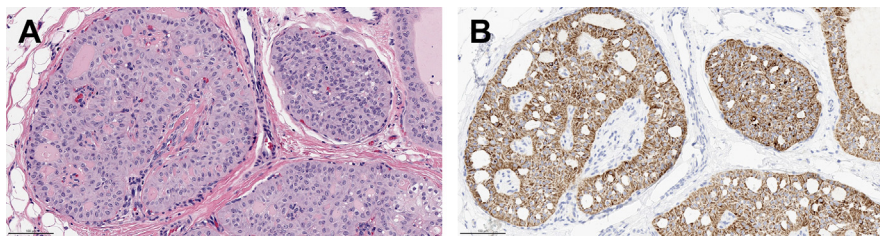

Fig. 4. Tall-cell carcinoma with reversed polarity. (*A*) The characteristic features of this tumor: solid papillary tumor cell nests, with cells of low-grade cytology and reversed polarity. (*B*) If needed, an IDH2 R172 immunostain can confirm the diagnosis.

TALL-CELL CARCINOMA WITH REVERSED POLARITY

Tall-cell carcinoma with reversed polarity is a relatively recently described entity characterized by an *IDH2* mutation (~80% of cases) and *PIK3CA* or *PIK3R1* mutations (up to 60% of cases).[24] These tumors most commonly occur in postmenopausal women and have an indolent clinical course, with lymph node metastases only rarely described. Microscopically, the tumor is composed of solid, papillary tumor cell nests of tall cells with reversed polarity (ie, the nucleus is located at the apical rather than the basal aspect of the cell), conferring a striking morphologic appearance (**Fig. 4**). The nuclei are low grade. The tumor is usually ER, PR, and HER2 negative; occasionally low ER positivity is reported.

With greater recognition of this tumor entity, the special histologic subtype should be provided so as to prevent the patient being treated as having a triple-negative breast cancer, NST. Mutation profiling may be used to identify the *IDH2 R172* mutation pathognomonic of this tumor. There is also an IDH2 R172 antibody that can be used for the immunohistochemical evaluation of this tumor (see **Fig. 4**).[25]

The limited data available suggest this tumor may be managed with surgical excision alone.

ADENOMYOEPITHELIOMA/MALIGNANT ADENOMYOEPITHELIOMA

Adenomyoepithelioma is a rare benign epithelial-myoepithelial tumor. Exceptionally, malignant transformation may occur. New data suggest that ER-negative adenomyoepitheliomas are more likely to harbor *HRAS* mutations, and it is these tumors that seem to have the greatest propensity for malignant transformation.[26,27] This observation suggests the possibility of using mutation profiling to identify which patients may be at risk for the development of carcinoma in this setting, thereby dictating a more stringent follow-up protocol. As with MYB and IDH2 R172, there is an antibody to NRAS Q61R that also recognizes HRAS Q61R and KRAS Q61R; however, experience with its use as a diagnostic or prognostic tool is limited at this time.[28]

ER-positive adenomyoepitheliomas are more likely to demonstrate *PIK3CA* mutations and do not seem to have the same risk as their ER-negative counterparts, albeit with limited data.[26]

PROGNOSTIC AND PREDICTIVE TESTING

For early-stage ER-positive/HER2-negative invasive breast carcinoma, the decision whether to give adjuvant chemotherapy hinges on the risk of distant recurrence. Several multigene assays have been developed to estimate this risk, including the 21-gene recurrence score assay (Oncotype Dx),[29] the 70-gene signature (MammaPrint),[30] the 50-gene assay (PAM50, Prosigna),[31] the 12-gene assay (EndoPredict),[32] and the Breast Cancer Index (BCI).[33] All assays are prognostic, providing an estimate of the risk of distant relapse. The 21-gene assay is both prognostic and predictive of chemotherapy benefit. These assays have been endorsed by the National Comprehensive Cancer Network (NCCN),[34] the American Society of Clinical Oncology (ASCO),[35–38] and St Gallen[39–41] guidelines for adjuvant treatment decisions in patients with early-stage, hormone receptor–positive breast cancer. The 21-gene assay is included in the prognostic staging in the AJCC Cancer Staging 8th Edition.[42] Of these multigene assays, the 21-gene recurrence score assay and the 70-gene signature assay are supported by level I clinical evidence, discussed later in detail.

21-Gene Recurrence Score Assay (Oncotype Dx)

The 21-gene recurrence score assay is a reverse transcriptase polymerase chain reaction (RT-PCR)-based test. The gene panel includes 16 cancer-related genes and 5 reference genes.[29] Derived recurrence scores range from 0 to 100, a higher score indicating a greater risk of recurrence. In the original publication, the cutoff points to classify low-, intermediate-, and high-risk groups were recurrence scores of less than 18, 18 to 30, and greater than or equal to 31, respectively.[29] These scores were later modified to recurrence scores of 0 to 10, 11 to 25, and greater than 25 in the prospective clinical trials.[43–45] The Trial Assigning Individualized Options for Treatment (TAILORx), a prospective randomized trial, found no benefit to chemotherapy in patients with early-stage ER-positive, HER2-negative, node-negative breast cancer with recurrence scores between 0 and 25, with the exception of young patients ($\leq$50 yrs) with recurrence scores of 16 to 25 who were shown to derive some benefit from chemotherapy.[43,44] The RxPONDER (Rx for Positive Node, Endocrine Responsive Breast Cancer) trial further validated the utility of the 21-gene recurrence score in patients with node-positive disease, demonstrating that postmenopausal patients with ER-positive, HER2-negative breast cancer with 1 to 3 positive lymph nodes and recurrence scores between 0 and 25 could be treated with endocrine therapy alone.[45] In contrast, premenopausal patients with 1 to 3 positive lymph nodes derived significant benefit from chemotherapy even in the setting of low recurrence scores.[45] The 21-gene recurrence score assay has had significant impact on adjuvant chemotherapy decisions (see separate article in this issue of the Clinics).

A significant association was also observed between the 21-gene recurrence score and the risk of locoregional recurrence (LRR) in both node-negative and node-positive patients.[46–48] The potential application of the 21-gene recurrence score for locoregional therapy decision-making in patients with early-stage ER-positive, HER2-negative breast cancer is under active investigation.

70-Gene Signature Assay (MammaPrint)

The 70-gene signature assay is a DNA microarray–based assay.[49] Multivariate analysis showed it to be an independent factor in predicting disease outcome in both patients with node-negative and those with node-positive breast cancer in a retrospective cohort.[30] Its clinical utility was validated in a prospective randomized phase 3 trial, the Microarray in Node-Negative and 1 to 3 Positive Lymph Node Disease May Avoid Chemotherapy (EORTC 10041/BIG 3-04 MINDACT).[50] The study assessed both the genomic risk (using the 70-gene signature) and the clinical risk (using a modified version of Adjuvant! Online). Patients with discordant clinical and genomic risks (low clinical risk/high genomic risk or high clinical risk/low genomic risk) were randomized to chemotherapy or no chemotherapy. It was found that patients with high clinical risk and low genomic risk had similar 5-year distant recurrence-free survival with or without adjuvant chemotherapy.[50] In patients with low clinical risk, genomic testing provided no added value as there was no significant benefit from the use of adjuvant chemotherapy regardless of genomic risk.[50] Thus, the 70-gene signature is of greatest value among patients with high clinical risk in whom its use led to a 46% reduction in the administration of adjuvant chemotherapy.[50]

The Integration of Genomic and Clinical Information in Prognostic Estimates

As described earlier for the MINDACT trial, genomic testing is best used in combination with clinicopathologic factors.[50] In fact, secondary analyses of the TAILORx trial found that incorporation of clinical risk stratification based on tumor size and

histologic grade added prognostic information to the 21-gene recurrence score.[51] A new tool, RSClin, which integrates the 21-gene recurrence score and selected clinical-pathological features (tumor grade, tumor size, and age), has been shown to provide more accurate prognostic information than recurrence score or clinicopathologic factors alone.[52]

Favorable Histologic Subtypes

Several special histologic subtypes of invasive breast cancer such as tubular carcinoma, cribriform carcinoma, pure mucinous carcinoma, encapsulated papillary carcinoma, and solid papillary carcinoma are associated with favorable prognoses. There are no data addressing whether multigene assays provide additional prognostic and predictive information in patients with these favorable histologic subtypes. Retrospective analysis of such cases has demonstrated the 21-gene recurrence scores to be lower than those of conventional invasive ductal carcinomas, high-risk recurrence scores being less frequently identified, in line with their favorable histology.[53,54] A high-risk recurrence score in any of these special histologic subtypes should prompt a careful pathologic review to confirm the diagnosis and identify tissue factors in the tumor sample that may have influenced the results.

Multigene Assays and Biomarker Assessment

ER, PR, and HER2 are among the 16 cancer-related genes assessed in the 21-gene assay. The 70-gene signature assay and PAM50 report gene expression–based "intrinsic" subtypes (luminal A, luminal B, HER2-enriched, and basal-like). However, these assays are not recommended as primary screening tests for biomarker assessment due to the lack of clinical validation supporting their utility in identifying patients for endocrine or HER2-targeted therapy. Validated immunohistochemistry (IHC) and/or in situ hybridization (ISH) remain the recommended standard tests for ER, PR, and HER2 in breast cancer according to ASCO/CAP guidelines.[55,56] Although a high concordance between standard IHC/ISH and the 21-gene RT-PCR assay for ER and PR status was observed,[57–60] a substantial false-negative rate for HER2 status by RT-PCR has been reported.[59–61] This discordance likely reflects a dilutional effect from contaminating nonneoplastic tissue such as normal breast epithelium, stroma, and tumor infiltrating lymphocytes, an inherent disadvantage of mRNA-based assays compared with IHC/ISH on intact tissue sections.

KI67

Although not genomic in nature, Ki67 assessment is briefly discussed here as a matter of interest.

Ki67 is a marker of cell proliferation. The Ki67 labeling index as assessed by immunohistochemistry is an established prognostic and predictive marker in early-stage breast cancer.[62] However, its clinical utility is limited due to the lack of interobserver and interlaboratory reproducibility and the lack of a standardized cutoff. A Ki67 index cutoff point of 14% was selected to distinguish between luminal A and luminal B breast cancer intrinsic subtype based on analysis of a cohort of breast cancers classified by PAM50.[63] The 14% cutoff was adopted by the 2011 St Gallen International Breast Cancer Consensus Guideline[64]; however, this was changed to 20% in the 2013 Guideline.[65] The 2021 St Gallen Consensus Conference endorsed the recent International Ki67 in Breast Cancer Working Group (IKWG) recommendation using Ki67 less than or equal to 5% (very low) or Ki67 greater than or equal to 30% (very high) to estimate prognosis and guide chemotherapy,[66] but more than one-third of the panel

voted "Ki67 threshold not known"[41], highlighting the lack of consensus that complicates use of Ki67 to guide therapy.

The IKWG recommendations set forth preanalytic requirements and a standardized visual scoring method to ensure uniform performance and interpretation of immunohistochemistry for Ki67.[66] Ki67 assessment is recommended only for hormone receptor–positive, HER2-negative early-stage breast cancer with Ki67 cutoffs of less than or equal to 5% or greater than or equal to 30%, as noted above. Even with careful attention to preanalytic issues and standardized scoring methods, there is still substantial interobserver/interlaboratory variability when Ki67 is in the greater than 5% and less than 30% range, limiting clinical applicability.

Recently, Ki67 assessment has been used to select patients for abemaciclib therapy. The Food and Drug Administration (FDA) approved abemaciclib, a CDK4/6 inhibitor, combined with endocrine therapy for hormone receptor-positive, HER2-negative, node-positive, high-risk early breast cancer with Ki67 greater than or equal to 20%. The approval was based on the MonarchE trial[67] but limited to a subset of patients with high recurrence risk and Ki67 greater than or equal to 20%. The FDA also approved the Ki-67 IHC MIB-1 pharmDx (Dako Omnis) assay as a companion diagnostic test for this indication. Updated analysis of the MonarchE study found Ki67 to be prognostic but not predictive.[68] Abemaciclib benefit was observed regardless of Ki67 status. The ASCO-updated recommendations broadened the application to patients with either 4 or more positive axillary lymph nodes or 1 to 3 positive axillary lymph nodes and either grade 3 disease, tumor size greater than or equal to 5 cm, or Ki67 greater then or equal to 20%, in keeping with the MonarchE trial design.[67]

For the aforementioned reasons, there is wide variation in utilization of Ki67 testing in breast cancer among pathology laboratories. Automated scoring by digital image analysis is still investigational but holds the promise of improving agreement and throughput, which is reported to take an average of 9 minutes/case using the IKWG recommended manual scoring method.

TUMOR-INFILTRATING LYMPHOCYTES

The prognostic and predictive value of tumor-infiltrating lymphocytes (TILs) is well established. An increased level of TILs is an independent predictor of response to neoadjuvant chemotherapy in all breast cancer subtypes.[69,70] In triple-negative breast cancer, high-level TILs are associated with better prognosis.[71,72] The presence of TILs is also associated with response to immunotherapy with programmed cell death ligand 1 (PD1/PD-L1) inhibitors. The percentage of stromal TILs is scored as the area of tumor stroma occupied by mononuclear inflammatory cells over total intratumoral stromal area, according to the recommendations by the International TILs Working Group.[73] The percentage of stromal TILs is a continuous variable, ranging from 0% to 100%. Every 10% increment in stromal TILs corresponds to an improved outcome.[69,72] Different studies used different cutoffs of stromal TILs in data analysis. Early studies used cutoffs of 60% or 50% TILs to define lymphocyte-predominant breast cancer.[69,71] Proposed TILs cutoffs of 30%, 20%, 10%, and even 5% have also been used.[74] There are currently no recommendations for a clinically relevant threshold and, therefore, scoring TILs is not implemented in daily practice outside of research or clinical trial settings.

TREATMENT DECISIONS

As noted in the introduction, genomic testing of breast cancers to identify actionable targets, with the exception of the few special histologic subtypes discussed earlier,

currently applies to the advanced stage or metastatic settings. Sequencing assays using tumor tissue are required to identify mutations, such as *ESR1, PIK3CA*, and *AKT1.* However, early detection evaluating circulating tumor cells may eliminate the need for a biopsy of the metastatic site (see later section and separate article in this issue of the Clinics).

Knowledge bases such as OncoKB that annotate somatic mutations for clinical significance offer the promise of personalized treatment options based on the cancer genome.[75]

IMMUNE CHECKPOINT INHIBITORS AND PD-L1 TESTING

Evaluation of the tumor for susceptibility to immune checkpoint inhibitors, such as pembrolizumab, is often requested in patients with advanced or metastatic triple-negative breast cancer. It is important to know that the drugs are approved for use in patients with tumors demonstrated to express PD-L1 using the appropriate FDA-approved companion diagnostic assay (**Table 1**). Each drug requires a different assay; each assay a specific vendor platform and antibody; and each antibody a different scoring system and different thresholds of positivity. Needless to say, this presents considerable challenges even for larger pathology laboratories in academic medical centers, as validation across platforms is not straightforward, and the relative infrequency of test interpretation makes maintaining proficiency and reproducibility difficult.[76,77] In spite of these hurdles, pathologists are committed to providing the information needed to care for patients with breast cancer, either with an in-house test option or by sending a tissue block to a reference laboratory. It is incumbent on both pathologists and oncologists to understand which test is indicated and to know what assay to order. That said, pembrolizumab was recently approved for high-risk early-stage triple-negative breast cancer in combination with chemotherapy as neoadjuvant treatment regardless of PD-L1 expression.[78]

Additional indicators of susceptibility to immune checkpoint inhibitors include tumor mutational burden, microsatellite instability, and mismatch repair defects. Most breast cancers have a low mutational burden, and microsatellite instability and mismatch repair defects are uncommon.[79] However, any opportunity to provide treatment benefit in patients with metastatic disease is invariably sought in the appropriate clinical setting. Tumor mutational burden information is provided with sequencing assays along with any specific somatic and/or genomic alterations present.

Table 1
Food and Drug Administration–approved companion diagnostic assays for programmed cell death ligand 1 in breast cancer

	SP142[a]	22C3 pharmDx
Immunotherapy	Atezolizumab	Pembrolizumab
Platform	Ventana BenchMark	DAKO
Scoring methods	Immune cells (IC)	Combined positive score (CPS)
Positivity definition	IC $\geq$ 1%	CPS$\geq$10
Clinical trial	IMpassion 130	KEYNOTE-355
Breast cancer subtype	Locally advanced or metastatic TNBC	Locally advanced or metastatic TNBC
Chemotherapy	Nab-paclitaxel	Taxane or gemcitabine-carboplatin

Abbreviation: TNBC, triple-negative breast cancer.
[a] Indication since withdrawn.

ESR1 MUTATIONS

The development of endocrine therapy resistance secondary to somatic alterations such as *ESR1* mutations in women with ER-positive metastatic breast cancer is a treatment challenge, particularly among those treated with aromatase inhibitors in this setting[80,81]; this seems to be less of an issue in primary ER-positive breast cancers, but emerging evidence suggests the presence of *ESR1* mutations in women with early-stage disease is associated with poorer disease-free and overall survival.[82] Despite being an established mechanism of endocrine resistance, *ESR1* mutations are not used as a biomarker to guide endocrine therapy, as current practice is to switch to fulvestrant after disease progression on aromatase inhibitors regardless of *ESR1* mutation status. The ASCO guideline does not recommend routine testing for *ESR1* mutations for hormonal receptor-positive, HER2-negative metastatic breast cancer.[83]

HER2 OVEREXPRESSION, AMPLIFICATION, AND MUTATION

HER2 overexpression and/or amplification is determined at the time of primary diagnosis, with approximately 15% to 20% of breast cancers being classified as HER2 "positive" and eligible for HER2-targeted therapies. Until recently, women with tumors lacking HER2 overexpression or amplification were ineligible for these therapeutic agents. However, emerging data demonstrating improved outcomes with the antibody drug conjugate trastuzumab deruxtecan (T-DXd) for patients with HER2 IHC 1+ and HER2 2+, fluorescence in situ hybridization nonamplified tumors have prompted reevaluation of how HER2 IHC-negative tumors are categorized, that is, the need for stricter attention to the separation of 0 and 1+ cases, as there are now treatment implications for this group.[84,85]

Further opportunities for HER2-targeted therapy in patients without demonstrated HER2 overexpression or amplification have been identified among patients with tumors harboring *HER2* mutations.[33] Activating HER2 mutations occur at a frequency of 2% to 3% overall in primary breast cancers with a particular preponderance seen in invasive lobular carcinomas (~8%). The pan-HER inhibitor neratinib has been shown to provide a clinical benefit rate of 31% in a pretreated population of patients with metastatic breast cancer.[33,86]

PIK3CA MUTATIONS FOR PI3K INHIBITOR TREATMENT

In the advanced stage or metastatic setting, identification of patients whose tumors harbor *PIK3CA* mutations offers the opportunity for treatment with the *PIK3CA* inhibitor, alpelisib, in combination with fulvestrant for hormonal receptor–positive/HER2-negative advanced breast cancer in postmenopausal women, or in male patients.[87] *PIK3CA* mutations are identified in up to 45% of patients with ER+, HER2-negative advanced breast cancer[88] and therefore offer a large potential pool of patients who may benefit from this therapy. Furthermore, trials exploring efficacy of alpelisib in patients with *PIK3CA*-mutated *HER2*+ breast cancer are ongoing.[89]

CIRCULATING TUMOR DNA

Circulating tumor DNA (ctDNA) is tumor-derived fragmented DNA present in the bloodstream. The sampling and analysis of ctDNA, also known as "liquid biopsy," offers a minimally invasive approach to genomic profiling and disease monitoring. The FDA approved the liquid biopsy next-generation sequencing (NGS)-based FoundationOne Liquid CDx test as a companion diagnostic device for specific indications, including the identification of *PIK3CA* mutations in breast cancer for treatment with alpelisib.[87]

The plasmaMATCH trial, a prospective trial evaluating the sensitivity of ctDNA to identify actionable mutations in advanced breast cancer, found that the agreement for mutation identification between ctDNA digital PCR and targeted sequencing using tissue biopsies was 96% to 99%.[90] Analytical validation of MSK-ACCESS (Memorial Sloan Kettering—Analysis of Circulating cfDNA to Examine Somatic Status), an institutional NGS platform for detection of somatic alterations in 129 genes in cell-free DNA, demonstrated 92% de novo sensitivity and 99% specificity.[91] Liquid biopsy does not replace tissue biopsy, given the importance of histology diagnosis, but potentially provides a valid alternative sampling strategy, especially when the metastatic site is not amendable for biopsy or a tissue sample obtained is not suitable for molecular analysis.

Multiple studies have demonstrated the potential utility of ctDNA in prognostication and in monitoring treatment response and disease progression in advanced breast cancer.[92–101] However, data are limited to retrospective analyses of prospective trials. In patients with early-stage breast cancer treated with neoadjuvant therapy, detection of ctDNA was associated with poor response and disease recurrence.[102,103] In a prospective multicenter study, detection of ctDNA was associated with relapse in early-stage breast cancer after ostensibly curative therapy, with ctDNA being detected at a median lead time of 10.7 months before clinical relapse.[104] There are no data to demonstrate that clinical intervention following early detection of molecular residual disease translates into improved patient outcome, limiting practical utility at this time. As such, current ASCO and NCCN guidelines do not recommend the use of ctDNA to guide adjuvant therapy in early-stage breast cancer or disease assessment and monitoring in the metastatic setting.[38]

THE UTILITY OF GENOMIC ANALYSIS IN RESOLVING DIAGNOSTIC DILEMMAS

In addition to identifying actionable somatic mutations and predisposing germline variants, genomic analysis can assist in diagnosis when definitive tumor classification cannot be accomplished based on histology and immunohistochemistry. A common clinical dilemma is the determination of primary tumor site in patients presenting with metastatic disease. For patients with a prior history of carcinoma, comparative genomic analysis of paired primary and metastatic tumor samples can determine whether a clonal relationship exists. Genomic comparison can also distinguish local recurrences from new primary carcinomas in patients with prior breast cancer. More challenging are situations in which the patient's history is noncontributory or a primary tumor sample is unavailable for comparison. Certain genomic alterations or combined patterns of mutation are associated with specific tumor types and help to predict tumor origin: examples include *APC* loss-of-function mutations in colorectal cancers, *TMPRSS2::ERG* fusions in prostate cancers, an ultraviolet-associated mutational signature of C > T substitutions in cutaneous melanomas, and the co-occurrence of *TP53* and *CTNNB1* mutations in endometrial cancer.[105] Breast carcinomas, except for certain special histologic subtypes, do not have unique genomic alterations. However, the absence of certain common mutations may be informative. For example, *EGRF* or *KRAS* mutations favor a carcinoma of pulmonary over mammary origin. Penson and colleagues reported a machine learning approach to the prediction of tumor type using genomic data.[105] The correct tumor type was predicted for 73.8% of 7791 patients in the training set and 74.1% of 11,644 patients in an independent cohort.[105] The performance was highest in tumor types with distinctive molecular profiles, such as uveal melanoma, glioma, and colorectal cancer, whereas lowest in esophagogastric, ovarian, and head and neck cancers due to

molecular heterogeneity among these tumors and the lack of distinguishing genomic alterations.[105] The algorithm identified carcinomas of mammary type with sensitivity and specificity values of 0.876 and 0.761, respectively, in 1181 patients with breast cancer included in the study cohort.[105]

SUMMARY

It is now well recognized that breast cancer is a heterogeneous disease. Optimal treatment is dictated by tumor biology and a multidisciplinary approach. Advances in genomics have further improved our understanding of breast cancer biology, with robust genomic assays becoming more easily accessible and being increasingly used in daily practice to assist in diagnosis, classification, risk stratification, and the detection of relevant germline mutations and actionable targets. For early-stage breast cancer, treatment is mainly informed by conventional clinicopathologic factors and biomarkers (ER, PR, HER2) using immunohistochemistry and/or in situ hybridization. Systemic therapy is largely driven by histology and receptor subtype. For early-stage hormonal receptor–positive, HER2-negative breast cancer, the use of multigene assays is well established for risk stratification and treatment escalation/deescalation. For advanced breast cancer, transcriptomic, genomic, epigenomic, and proteomic landscapes may inform personalized treatment options. The translation of such data into individualized treatment plans conferring survival benefits is the challenge ahead.

REFERENCES

1. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72:1117–30.
2. Robson M, Im SA, Senkus E, et al. Olaparib for Metastatic Breast Cancer in Patients with a Germline BRCA Mutation. N Engl J Med 2017;377:523–33.
3. Robson ME, Tung N, Conte P, et al. OlympiAD final overall survival and tolerability results: Olaparib versus chemotherapy treatment of physician's choice in patients with a germline BRCA mutation and HER2-negative metastatic breast cancer. Ann Oncol 2019;30:558–66.
4. Litton JK, Rugo HS, Ettl J, et al. Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation. N Engl J Med 2018;379:753–63.
5. Litton JK, Hurvitz SA, Mina LA, et al. Talazoparib versus chemotherapy in patients with germline BRCA1/2-mutated HER2-negative advanced breast cancer: final overall survival results from the EMBRACA trial. Ann Oncol 2020;31:1526–35.
6. Tutt ANJ, Garber JE, Kaufman B, et al. Adjuvant Olaparib for Patients with BRCA1- or BRCA2-Mutated Breast Cancer. N Engl J Med 2021;384:2394–405.
7. Mandelker D, Zhang L, Kemel Y, et al. Mutation Detection in Patients With Advanced Cancer by Universal Sequencing of Cancer-Related Genes in Tumor and Normal DNA vs Guideline-Based Germline Testing. Jama 2017;318:825–35.
8. Stadler ZK, Maio A, Chakravarty D, et al. Therapeutic Implications of Germline Testing in Patients With Advanced Cancers. J Clin Oncol 2021;39:2698–709.
9. Hornick JL. Replacing Molecular Genetic Testing With Immunohistochemistry Using Antibodies That Recognize the Protein Products of Gene Rearrangements: "Next-generation" Immunohistochemistry. Am J Surg Pathol 2021;45:584–6.

10. Shin SJ, Rosen PP. Solid variant of mammary adenoid cystic carcinoma with basaloid features: a study of nine cases. Am J Surg Pathol 2002;26:413–20.
11. D'Alfonso TM, Mosquera JM, MacDonald TY, et al. MYB-NFIB gene fusion in adenoid cystic carcinoma of the breast with special focus paid to the solid variant with basaloid features. Hum Pathol 2014;45:2270–80.
12. Slodkowska E, Xu B, Kos Z, et al. Predictors of Outcome in Mammary Adenoid Cystic Carcinoma: A Multi-Institutional Study. Am J Surg Pathol 2020;44:214–23.
13. Massé J, Truntzer C, Boidot R, et al. Solid-type adenoid cystic carcinoma of the breast, a distinct molecular entity enriched in NOTCH and CREBBP mutations. Mod Pathol 2020;33:1041–55.
14. Schwartz CJ, Brogi E, Marra A, et al. The clinical behavior and genomic features of the so-called adenoid cystic carcinomas of the solid variant with basaloid features. Mod Pathol 2022;35:193–201.
15. Persson M, Andrén Y, Mark J, et al. Recurrent fusion of MYB and NFIB transcription factor genes in carcinomas of the breast and head and neck. Proc Natl Acad Sci U S A 2009;106:18740–4.
16. Kim J, Geyer FC, Martelotto LG, et al. MYBL1 rearrangements and MYB amplification in breast adenoid cystic carcinomas lacking the MYB-NFIB fusion gene. J Pathol 2018;244:143–50.
17. West RB, Kong C, Clarke N, et al. MYB expression and translocation in adenoid cystic carcinomas and other salivary gland tumors with clinicopathologic correlation. Am J Surg Pathol 2011;35:92–9.
18. Brill LB 2nd, Kanner WA, Fehr A, et al. Analysis of MYB expression and MYB-NFIB gene fusions in adenoid cystic carcinoma and other salivary neoplasms. Mod Pathol 2011;24:1169–76.
19. Tognon C, Knezevich SR, Huntsman D, et al. Expression of the ETV6-NTRK3 gene fusion as a primary event in human secretory breast carcinoma. Cancer Cell 2002;2:367–76.
20. Hechtman JF, Benayed R, Hyman DM, et al. Pan-Trk Immunohistochemistry Is an Efficient and Reliable Screen for the Detection of NTRK Fusions. Am J Surg Pathol 2017;41:1547–51.
21. Harrison BT, Fowler E, Krings G, et al. Pan-TRK Immunohistochemistry: A Useful Diagnostic Adjunct For Secretory Carcinoma of the Breast. Am J Surg Pathol 2019;43:1693–700.
22. Hoda RS, Brogi E, Pareja F, et al. Secretory carcinoma of the breast: clinicopathologic profile of 14 cases emphasising distant metastatic potential. Histopathology 2019;75:213–24.
23. Shukla N, Roberts SS, Baki MO, et al. Successful Targeted Therapy of Refractory Pediatric ETV6-NTRK3 Fusion-Positive Secretory Breast Carcinoma. JCO Precis Oncol 2017;2017.
24. Chiang S, Weigelt B, Wen HC, et al. IDH2 Mutations Define a Unique Subtype of Breast Cancer with Altered Nuclear Polarity. Cancer Res 2016;76:7118–29.
25. Pareja F, da Silva EM, Frosina D, et al. Immunohistochemical analysis of IDH2 R172 hotspot mutations in breast papillary neoplasms: applications in the diagnosis of tall cell carcinoma with reverse polarity. Mod Pathol 2020;33:1056–64.
26. Geyer FC, Li A, Papanastasiou AD, et al. Recurrent hotspot mutations in HRAS Q61 and PI3K-AKT pathway genes as drivers of breast adenomyoepitheliomas. Nat Commun 2018;9:1816.
27. Watanabe S, Otani T, Iwasa T, et al. A Case of Metastatic Malignant Breast Adenomyoepithelioma With a Codon-61 Mutation of HRAS. Clin Breast Cancer 2019;19:e589–92.

28. Pareja F, Toss MS, Geyer FC, et al. Immunohistochemical assessment of HRAS Q61R mutations in breast adenomyoepitheliomas. Histopathology 2020;76:865–74.
29. Paik S, Shak S, Tang G, et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med 2004;351:2817–26.
30. van de Vijver MJ, He YD, van't Veer LJ, et al. A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 2002;347:1999–2009.
31. Parker JS, Mullins M, Cheang MC, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27:1160–7.
32. Filipits M, Rudas M, Jakesz R, et al. A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. Clin Cancer Res 2011;17:6012–20.
33. Ma XJ, Salunga R, Dahiya S, et al. A five-gene molecular grade index and HOXB13:IL17BR are complementary prognostic factors in early stage breast cancer. Clin Cancer Res 2008;14:2601–8.
34. Gradishar WJ, Moran MS, Abraham J et al. Breast Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw. 20, 2022, 691-722; Accessed July 2022.
35. Harris LN, Ismaila N, McShane LM, et al. Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol 2016;34:1134–50.
36. Krop I, Ismaila N, Andre F, et al. Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline Focused Update. J Clin Oncol 2017;35:2838–47.
37. Andre F, Ismaila N, Henry NL, et al. Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: ASCO Clinical Practice Guideline Update-Integration of Results From TAILORx. J Clin Oncol 2019;37:1956–64.
38. Andre F, Ismaila N, Allison KH, et al. Biomarkers for Adjuvant Endocrine and Chemotherapy in Early-Stage Breast Cancer: ASCO Guideline Update. J Clin Oncol 2022;40:1816–37.
39. Coates AS, Winer EP, Goldhirsch A, et al. Tailoring therapies–improving the management of early breast cancer: St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2015. Ann Oncol 2015;26:1533–46.
40. Burstein HJ, Curigliano G, Loibl S, et al. Estimating the benefits of therapy for early-stage breast cancer: the St. Gallen International Consensus Guidelines for the primary therapy of early breast cancer 2019. Ann Oncol 2019;30:1541–57.
41. Burstein HJ, Curigliano G, Thürlimann B, et al. Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. Ann Oncol 2021;32:1216–35.
42. Amin MB, Greene FL, Edge SB, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin 2017;67:93–9.
43. Sparano JA, Gray RJ, Makower DF, et al. Prospective Validation of a 21-Gene Expression Assay in Breast Cancer. N Engl J Med 2015;373:2005–14.

44. Sparano JA, Gray RJ, Makower DF, et al. Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. N Engl J Med 2018;379:111–21.
45. Kalinsky K, Barlow WE, Gralow JR, et al. 21-Gene Assay to Inform Chemotherapy Benefit in Node-Positive Breast Cancer. N Engl J Med 2021;385:2336–47.
46. Mamounas EP, Tang G, Fisher B, et al. Association between the 21-gene recurrence score assay and risk of locoregional recurrence in node-negative, estrogen receptor-positive breast cancer: results from NSABP B-14 and NSABP B-20. J Clin Oncol 2010;28:1677–83.
47. Mamounas EP, Liu Q, Paik S, et al. 21-Gene Recurrence Score and Locoregional Recurrence in Node-Positive/ER-Positive Breast Cancer Treated With Chemo-Endocrine Therapy. J Natl Cancer Inst 2017;109.
48. Turashvili G, Chou JF, Brogi E, et al. 21-Gene recurrence score and locoregional recurrence in lymph node-negative, estrogen receptor-positive breast cancer. Breast Cancer Res Treat 2017;166:69–76.
49. van 't Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature 2002;415:530–6.
50. Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. N Engl J Med 2016;375:717–29.
51. Sparano JA, Gray RJ, Ravdin PM, et al. Clinical and Genomic Risk to Guide the Use of Adjuvant Therapy for Breast Cancer. N Engl J Med 2019;380:2395–405.
52. Sparano JA, Crager MR, Tang G, et al. Development and Validation of a Tool Integrating the 21-Gene Recurrence Score and Clinical-Pathological Features to Individualize Prognosis and Prediction of Chemotherapy Benefit in Early Breast Cancer. J Clin Oncol 2021;39:557–64.
53. Turashvili G, Brogi E, Morrow M, et al. The 21-gene recurrence score in special histologic subtypes of breast cancer with favorable prognosis. Breast Cancer Res Treat 2017;165:65–76.
54. Tadros AB, Wen HY, Morrow M. Breast Cancers of Special Histologic Subtypes Are Biologically Diverse. Ann Surg Oncol 2018;25:3158–64.
55. Allison KH, Hammond MEH, Dowsett M, et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update. J Clin Oncol 2020;38:1346–66.
56. Wolff AC, Hammond MEH, Allison KH, et al. Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol 2018;36:2105–22.
57. O'Connor SM, Beriwal S, Dabbs DJ, et al. Concordance between semiquantitative immunohistochemical assay and oncotype DX RT-PCR assay for estrogen and progesterone receptors. Appl Immunohistochem Mol Morphol 2010;18:268–72.
58. Kraus JA, Dabbs DJ, Beriwal S, et al. Semi-quantitative immunohistochemical assay versus oncotype DX(®) qRT-PCR assay for estrogen and progesterone receptors: an independent quality assurance study. Mod Pathol 2012;25:869–76.
59. Park MM, Ebel JJ, Zhao W, et al. ER and PR immunohistochemistry and HER2 FISH versus oncotype DX: implications for breast cancer treatment. Breast J 2014;20:37–45.
60. Neely C, You S, Mendoza PM, et al. Comparing breast biomarker status between routine immunohistochemistry and FISH studies and Oncotype DX testing, a study of 610 cases. Breast J 2018;24:889–93.

61. Dabbs DJ, Klein ME, Mohsin SK, et al. High false-negative rate of HER2 quantitative reverse transcription polymerase chain reaction of the Oncotype DX test: an independent quality assurance study. J Clin Oncol 2011;29:4279–85.
62. Yerushalmi R, Woods R, Ravdin PM, et al. Ki67 in breast cancer: prognostic and predictive potential. Lancet Oncol 2010;11:174–83.
63. Cheang MC, Chia SK, Voduc D, et al. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. J Natl Cancer Inst 2009;101:736–50.
64. Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes–dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. Ann Oncol 2011;22:1736–47.
65. Goldhirsch A, Winer EP, Coates AS, et al. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Ann Oncol 2013;24:2206–23.
66. Nielsen TO, Leung SCY, Rimm DL, et al. Assessment of Ki67 in Breast Cancer: Updated Recommendations From the International Ki67 in Breast Cancer Working Group. J Natl Cancer Inst 2021;113:808–19.
67. Johnston SRD, Harbeck N, Hegg R, et al. Abemaciclib Combined With Endocrine Therapy for the Adjuvant Treatment of HR+, HER2-, Node-Positive, High-Risk, Early Breast Cancer (monarchE). J Clin Oncol 2020;38:3987–98.
68. Harbeck N, Rastogi P, Martin M, et al. Adjuvant abemaciclib combined with endocrine therapy for high-risk early breast cancer: updated efficacy and Ki-67 analysis from the monarchE study. Ann Oncol 2021;32:1571–81.
69. Denkert C, Loibl S, Noske A, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 2010;28:105–13.
70. Denkert C, von Minckwitz G, Darb-Esfahani S, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol 2018;19:40–50.
71. Loi S, Sirtaine N, Piette F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. J Clin Oncol 2013;31:860–7.
72. Loi S, Drubay D, Adams S, et al. Tumor-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers. J Clin Oncol 2019;37:559–69.
73. Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Ann Oncol 2015;26:259–71.
74. Loi S, Michiels S, Adams S, et al. The journey of tumor-infiltrating lymphocytes as a biomarker in breast cancer: clinical utility in an era of checkpoint inhibition. Ann Oncol 2021;32:1236–44.
75. Chakravarty D, Gao J, Phillips SM, et al. OncoKB: A Precision Oncology Knowledge Base. JCO Precis Oncol 2017;2017.
76. Han G, Schell MJ, Reisenbichler ES, et al. Determination of the number of observers needed to evaluate a subjective test and its application in two PD-L1 studies. Stat Med 2022;41:1361–75.

77. Reisenbichler ES, Han G, Bellizzi A, et al. Prospective multi-institutional evaluation of pathologist assessment of PD-L1 assays for patient selection in triple negative breast cancer. Mod Pathol 2020;33:1746–52.
78. Schmid P, Cortes J, Pusztai L, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. N Engl J Med 2020;382:810–21.
79. Sajjadi E, Venetis K, Piciotti R, et al. Mismatch repair-deficient hormone receptor-positive breast cancers: Biology and pathological characterization. Cancer Cell Int 2021;21:266.
80. Schiavon G, Hrebien S, Garcia-Murillas I, et al. Analysis of ESR1 mutation in circulating tumor DNA demonstrates evolution during therapy for metastatic breast cancer. Sci Transl Med 2015;7:313ra182.
81. Clatot F, Perdrix A, Beaussire L, et al. Risk of early progression according to circulating ESR1 mutation, CA-15.3 and cfDNA increases under first-line anti-aromatase treatment in metastatic breast cancer. Breast Cancer Res 2020; 22:56.
82. Dahlgren M, George AM, Brueffer C, et al. Preexisting Somatic Mutations of Estrogen Receptor Alpha (ESR1) in Early-Stage Primary Breast Cancer. JNCI Cancer Spectr 2021;5.
83. Burstein HJ, Somerfield MR, Barton DL, et al. Endocrine Treatment and Targeted Therapy for Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Metastatic Breast Cancer: ASCO Guideline Update. J Clin Oncol 2021;39:3959–77.
84. Modi S, Park H, Murthy RK, et al. Antitumor Activity and Safety of Trastuzumab Deruxtecan in Patients With HER2-Low-Expressing Advanced Breast Cancer: Results From a Phase Ib Study. J Clin Oncol 2020;38:1887–96.
85. Modi S, Jacot W, Yamashita T, et al. Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer. N Engl J Med 2022;387:9–20.
86. Hyman DM, Piha-Paul SA, Won H, et al. HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature 2018;554:189–94.
87. André F, Ciruelos E, Rubovszky G, et al. Alpelisib for PIK3CA-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. N Engl J Med 2019;380:1929–40.
88. Martínez-Sáez O, Chic N, Pascual T, et al. Frequency and spectrum of PIK3CA somatic mutations in breast cancer. Breast Cancer Res 2020;22:45.
89. Pérez-Fidalgo JA, Criscitiello C, Carrasco E, et al. A phase III trial of alpelisib + trastuzumab ± fulvestrant versus trastuzumab + chemotherapy in HER2+ PIK3CA-mutated breast cancer. Future Oncol 2022;18:2339–49.
90. Turner NC, Kingston B, Kilburn LS, et al. Circulating tumour DNA analysis to direct therapy in advanced breast cancer (plasmaMATCH): a multicentre, multicohort, phase 2a, platform trial. Lancet Oncol 2020;21:1296–308.
91. Rose Brannon A, Jayakumaran G, Diosdado M, et al. Enhanced specificity of clinical high-sensitivity tumor mutation profiling in cell-free DNA via paired normal sequencing using MSK-ACCESS. Nat Commun 2021;12:3770.
92. Dawson SJ, Tsui DW, Murtaza M, et al. Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med 2013;368:1199–209.
93. Fribbens C, Garcia Murillas I, Beaney M, et al. Tracking evolution of aromatase inhibitor resistance with circulating tumour DNA analysis in metastatic breast cancer. Ann Oncol 2018;29:145–53.
94. O'Leary B, Hrebien S, Morden JP, et al. Early circulating tumor DNA dynamics and clonal selection with palbociclib and fulvestrant for breast cancer. Nat Commun 2018;9:896.

95. O'Leary B, Cutts RJ, Liu Y, et al. The Genetic Landscape and Clonal Evolution of Breast Cancer Resistance to Palbociclib plus Fulvestrant in the PALOMA-3 Trial. Cancer Discov 2018;8:1390–403.
96. Paoletti C, Schiavon G, Dolce EM, et al. Circulating Biomarkers and Resistance to Endocrine Therapy in Metastatic Breast Cancers: Correlative Results from AZD9496 Oral SERD Phase I Trial. Clin Cancer Res 2018;24:5860–72.
97. Hrebien S, Citi V, Garcia-Murillas I, et al. Early ctDNA dynamics as a surrogate for progression-free survival in advanced breast cancer in the BEECH trial. Ann Oncol 2019;30:945–52.
98. O'Leary B, Cutts RJ, Huang X, et al. Circulating Tumor DNA Markers for Early Progression on Fulvestrant With or Without Palbociclib in ER+ Advanced Breast Cancer. J Natl Cancer Inst 2021;113:309–17.
99. Kingston B, Cutts RJ, Bye H, et al. Genomic profile of advanced breast cancer in circulating tumour DNA. Nat Commun 2021;12:2423.
100. Bardia A, Su F, Solovieff N, et al. Genomic Profiling of Premenopausal HR+ and HER2- Metastatic Breast Cancer by Circulating Tumor DNA and Association of Genetic Alterations With Therapeutic Response to Endocrine Therapy and Ribociclib. JCO Precis Oncol 2021;5.
101. Tolaney SM, Toi M, Neven P, et al. Clinical Significance of PIK3CA and ESR1 Mutations in Circulating Tumor DNA: Analysis from the MONARCH 2 Study of Abemaciclib plus Fulvestrant. Clin Cancer Res 2022;28:1500–6.
102. Radovich M, Jiang G, Hancock BA, et al. Association of Circulating Tumor DNA and Circulating Tumor Cells After Neoadjuvant Chemotherapy With Disease Recurrence in Patients With Triple-Negative Breast Cancer: Preplanned Secondary Analysis of the BRE12-158 Randomized Clinical Trial. JAMA Oncol 2020;6:1410–5.
103. Magbanua MJM, Swigart LB, Wu HT, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. Ann Oncol 2021;32:229–39.
104. Garcia-Murillas I, Chopra N, Comino-Méndez I, et al. Assessment of Molecular Relapse Detection in Early-Stage Breast Cancer. JAMA Oncol 2019;5:1473–8.
105. Penson A, Camacho N, Zheng Y, et al. Development of Genome-Derived Tumor Type Prediction to Inform Clinical Cancer Care. JAMA Oncol 2020;6:84–91.