New Developments in Breast Cancer and Their Impact on Daily Practice in Pathology

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• Advances in research have transformed our understanding of breast cancers and have altered the daily practice of pathology. Theranostic evaluations performed by pathologists are now critical in triaging the patients into appropriate treatment groups, as are new guidelines that were recently established for the evaluation of HER2/neu gene amplification. Emerging molecular classifications of breast cancers bring novel perspectives to the assessment of individual cases, and opportunities for better treatments. Molecular studies have particularly shed light on distinct biological subsets of triple-negative breast cancers, for which new targeted therapies are being developed. The prognostic and therapeutic utility of new histopathologic parameters, such as tumor-infiltrating lymphocytes, are also being elucidated, and new protocols have been devised for the pathologic evaluation of breast specimens that have undergone neoadjuvant treatment. Novel clinical practices, such as radioactive seed localization, also affect the way breast specimens are processed and evaluated. In this brief review, we highlight the developments that are most relevant to pathology and are changing or could potentially impact our daily practice.

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Breast carcinoma is the most commonly diagnosed cancer in women. It is estimated that approximately 15% of women will develop breast cancer during their lifetime. In the past few years, significant developments have taken place in our understanding and classification of breast cancer, some of which are incorporated into the most recent American Society of Clinical Oncology (ASCO) recommendations. These recent developments, which have profound implications in breast cancer diagnosis and treatments and are rapidly being translated into daily practice, will be discussed in this review.

MOLECULAR AND IMMUNOPHENOTYPIC CLASSIFICATION OF BREAST CARCINOMA

The seminal studies by Perou et al4 and Sorlie et al5 classified breast carcinoma into at least 4 distinct intrinsic subtypes by molecular phenotype: luminal A, luminal B, HER2/neu enriched, and basal-like. Luminal A and B breast cancers are estrogen receptor (ER) or progesterone receptor (PR) positive, while the luminal B subtype is positive for HER2/neu gene amplification or shows a high proliferative index.3,4 The HER2/neu-enriched subtype is negative for ER and PR expression and positive for HER2/neu gene amplification. Most breast carcinomas fall into these 3 groups, for which specific targeted therapies are available.

The basal-like subtype is characterized by high expression of keratins 5, 6, and 17, which are usually found in breast myoepithelial cells (and the basal epithelial cells of other normal tissues).1,5–11 This subtype is associated with a higher frequency of p53 mutation, higher proliferative index, higher tumor grade, and worse prognosis, and it occurs more frequently in African Americans.1,3,5–10,12–17 Most BRCA-mutated breast carcinomas fall into this subtype.1,18,19

In clinical practice, the breast carcinomas that are negative for ER, PR, and HER2/neu are referred to as triple-negative breast carcinomas (TNBCs). Most basal-like carcinomas are TNBC.5–8 Studies have shown that most TNBCs with positive cytokeratin 5/6 and epidermal growth factor receptor (EGFR) expression correspond to the basal-like subtype.5,6,8 It should be noted, however, that although there is great overlap between basal-like carcinoma and TNBC, these 2 entities are not exactly the same: the basal-like subtype is a molecular classification with a distinct gene signature, while TNBC is a clinical classification defined by negative ER, PR, and HER2/neu expression. Approximately 50% to 80% of TNBCs are basal-like, but 10% to 30% are HER2/neu enriched and a small number are luminal.1,2,20

Triple-negative breast carcinoma is also characterized by loss of various markers that are otherwise commonly expressed in low-grade breast cancers and used in daily diagnostic practice, including GATA-3 expression.21

Recent studies revealed the existence of another subtype, the claudin-low subtype,22 characterized by down-regulation of tight junction proteins including E-cadherin, occludin, and some claudins, and by high expression of genes associated with epithelial-mesenchymal transition (EMT), immune response, and the breast cancer stem-cell phenotype.22,23 The claudin-low breast carcinoma is generally triple negative and this subtype includes most of the spindle cell (sarcomatoid) metaplastic carcinomas.24,25 The
enhanced expression of EMT and breast cancer stem cell genes. The sixth, or luminal androgen receptor (LAR) group, is enriched in hormone regulated pathways with high androgen receptor (AR) expression both at the mRNA and protein levels, but this group also shows the intrinsic luminal gene signature and has a low proliferative rate. In a separate study, Burstein et al identified 4 molecular subtypes in TNBC, which showed some overlaps with those reported by Lehmann et al, but these 2 classifications are not identical (Table).

The TNBC subtypes described by Lehmann et al have different clinical outcomes and varying responses to therapy. For example, the LAR subtype had significantly worse relapse-free survival than BL1, IM, and MSL subtypes. The M subtype had significantly worse relapse-free survival than the BL1 and IM subtypes. Masuda et al showed that the BL1 subtype had the highest, and BL2 and LAR the lowest, pathologic complete response rates (52%, 0%, and 10%, respectively) when treated with neoadjuvant chemotherapy; however, despite having a low pathologic complete response rate, the LAR subtype had the best overall survival.

Genomic studies on TNBC have identified many targetable pathways. Shah et al identified mutations in PIK3CA, EGFR, RB1, PTEN, and in other genes including the newly identified deletions of the PARK2 tumor suppressor gene in TNBC. Other groups also found other targetable pathways in TNBCs, such as FGR2 gene amplification.

These classification schemes illustrate that the TNBCs are heterogeneous, comprising several theranostically distinct subsets. These classifications will likely become important in better management of affected patients in the near future, although they have not yet taken a specific role in pathologists’ daily practice.

**MOLECULAR CHARACTERIZATION OF TRIPLE-NEGATIVE BREAST CANCERS**

Triple-negative breast carcinoma remains highly challenging in terms of diagnosis and treatment. The main tasks are to predict the behavior of individual tumors in this group and to identify targetable pathways. Molecular studies have attempted to better subclassify the TNBCs. Lehmann et al identified 6 defined subtypes of TNBC as well as a seventh, unstable subtype. In this classification, the basal-like–1 (BL1) group is defined by higher expression of proliferative and DNA damage repair genes. The basal-like–2 (BL2) group, by contrast, is enriched for growth factor pathways and shows basal/myoepithelial features such as upregulation of TP53 and CD10. Most BL1 and some BL2 breast cancers belong to the intrinsic basal-like subtype and are associated with BRCA mutations. The third group is the immunomodulatory (IM) group, defined by expression of immune-related genes; this group also encompasses medullary carcinoma, which has a good prognosis. The fourth and fifth categories, termed mesenchymal (M) and mesenchymal stem-cell-like (MSL), respectively, are similar to the claudin-low subtype in showing enhanced expression of EMT and breast cancer stem cell genes. The sixth, or luminal androgen receptor (LAR) group, is enriched in hormone regulated pathways with high androgen receptor (AR) expression both at the mRNA and protein levels, but this group also shows the intrinsic luminal gene signature and has a low proliferative rate. In a separate study, Burstein et al identified 4 molecular subtypes in TNBC, which showed some overlaps with those reported by Lehmann et al, but these 2 classifications are not identical (Table).

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**EVOLVING TARGETED THERAPIES IN TRIPLE-NEGATIVE BREAST CANCER**

The following is a brief overview of the most salient recent discoveries of the pathways with targetable agents in TNBCs. Although there are no established guidelines yet as to their incorporation to daily practice, it is safe to assume that at least some will become a part of routine evaluation of breast carcinomas in the near future.
Expression associated with promoter methylation. Sev-
expressions are frequent in ER- and HER2/neu-positive cancers and may account for treatment resistance in these tumors. On the other hand, while are frequent in TNBCs, they have been reported in up to 47% of metaplastic carcinomas of the breast. Thus, it is important to recognize and classify metaplastic carcinomas accurately, rather than designating them merely as poorly differentiated carcinomas. Several trials are underway to determine the role of mTOR and PI3K pathway inhibitors in breast cancer.

Androgen Receptor

A significant number of TNBCs show AR overexpression. In vitro studies showed sensitivity of LAR cell lines and of cell-line–derived xenografts to the AR inhibitor bicalutamide. A phase II trial showed a good safety profile of bicalutamide and a 19% benefit rate in patients with advanced AR-positive, ER/PR-negative breast cancer. If AR inhibition eventually proves to be an effective therapy, theranostic evaluation of AR expression will likely become a part of routine practice, on a par with ER, PR, and HER2.

Platinum-Based Chemotherapy and Poly-ADP Ribose Polymerase Inhibitor

Platinum and poly-ADP ribose polymerase (PARP) inhibitors are agents that disrupt chromosomal DNA integrity and might be useful in treating tumors with DNA repair deficiency. Platinum-based therapy has been used in clinical trials to treat TNBC and has shown benefits in patients with BRCA1 germline mutations or low BRCA1 expression associated with promoter methylation. Several clinical trials also examined the safety and efficacy of PARP inhibitors in TNBC. A subset of TNBCs with DNA repair deficiency, such as the Lehmann BL1 and BL2 subtypes, or TNBC with BRCA mutations, may also potentially benefit from these reagents.

p53 Pathway

TP53 is the most frequently mutated gene in cancer, and its product, p53, is an important tumor suppressor protein. Abnormalities of p53 lead to tumor initiation and progression. Shah et al showed that 62% of basal-like and 43% of non–basal-like TNBCs had TP53 mutations. Several clinical trials are now examining the antitumor function of small molecules that target the p53 pathway.

Growth Factor Pathways

Molecular studies have revealed alterations of several growth factor pathways in TNBC. The Lehmann BL2 and MSL subtypes are enriched in growth factor–related gene expression. Epidermal growth factor receptor is frequently expressed in TNBC. Anti-EGFR antibodies have been tested in early clinical trials in metastatic TNBC. Antagonists of the vascular endothelial growth factor receptor and fibroblast growth factor receptor are also under investigation. While none of these markers is currently tested routinely, it is conceivable that in the near future, some of these markers will be used to better select TNBC treatments.

Immune Checkpoint Inhibitors

The programmed cell death protein 1 (PD-1) is an immune checkpoint protein that is expressed on immune cells. When PD-1 binds to its ligand (PD-L1), PD-1 suppresses T-cell immune functions. Anti–PD-1 pathway antibodies have been approved by the US Food and Drug Administration (FDA) to treat various cancers. Early clinical trials are investigating the efficacy of anti–PD-1 pathway antibodies in TNBC. Our unpublished data show that about 50% of TNBCs have positive PD-L1 staining in tumor or intratumoral stromal cells. If PD-L1 antibody proves an effective treatment, evaluation of PD-L1 expression will undoubtedly be required in breast cancers.

In summary, extensive research and clinical trials are underway investigating targeted therapies in treating TNBC, and some of these are already becoming used in daily practice, such as the platinum-based agents. Accurate morphologic classification, supplemented by immunohistochemical and molecular studies, is crucial in directing patients into the most appropriate treatment protocols. We, as pathologists, have to embrace these new developments and be prepared to incorporate the new biomarkers, as data warrant, into our morphologic evaluation of breast cancers.

UPDATED APPROACH TO THE EVALUATION OF HER2/neu

Evaluation of HER2/neu expression, along with ER/PR, is crucial in prognostication and therapeutic stratification of breast cancers. ASCO/College of American Pathologists (CAP) guidelines mandate routine testing of HER2/neu, along with ER and PR, in every new, recurrent, invasive, and metastatic breast carcinoma. The guidelines were revised in 2013 to improve the testing sensitivity and accuracy, since inaccuracy in HER2/neu testing was found to be as high as 20%. Misinterpretation of HER2/neu expression can lead to very expensive medical treatments as well as exposure of the patient to unnecessary treatment with the associated risk on one hand, or missed opportunity to potentially cure a patient on the other. The new guidelines emphasize sensitivity over specificity, especially after pertuzumab received FDA approval for the neoadjuvant treatment of patients with HER2/neu+ breast cancer, and as optimizing treatment of HER2/neu-positive metastatic breast cancer.

The 2013 HER2/neu scoring criteria by IHC are summarized as follows.

- Score 0: No staining observed or membrane staining that is incomplete and faint/barely perceptible and within 10% or less of the invasive tumor cells. Score 0 is negative for HER2/neu overexpression.
- Score 1+: Incomplete membrane staining that is faint/barely perceptible and within more than 10% of invasive tumor cells. Score 1+ is negative for HER2/neu overexpression.
Score 2+: Circumferential membrane staining that is incomplete and/or weak/moderate and within more than 10% of the invasive tumor cells, or complete and circumferential membrane staining that is intense and within 10% or less of the invasive tumor cells. Score 2+ is equivocal and requires reflex testing by in situ hybridization for HER2/neu amplification.

Score 3+: Complete intense circumferential membrane staining in more than 10% of the invasive tumor cells. Score 3+ is positive for HER2/neu overexpression.

Three important points about these scoring criteria need to be emphasized: (1) To render a 3+ score, completely circumferential and very strong membranous staining is required (Figure 1, A). In our consultation practice, we see examples with strong but granular cytoplasmic staining that were initially overcalled as 3+ only to prove negative by fluorescence in situ hybridization (FISH) analysis. For similar reasons, some of the automated systems also tend to overcall these 2+ cases as 3+ (Figure 1, B), which creates unwarranted treatment dilemma. (2) It was realized that using the terms circumferential and incomplete together in defining score 2+ was confusing, resulting in large numbers of cases being submitted for reflex in situ hybridization testing. Such testing would increase the cost of the test and be a resource issue for many laboratories. Therefore, the 2013 guidelines are being updated. The definition for HER2/neu IHC 2+ will be “weak to moderate complete membrane staining that is observed in more than 10% of tumor cells.” (3) The 2013 guidelines stated that, “If the initial HER2 test in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test may be ordered on the excision specimen.” These revisions are expected to be published shortly.

For HER2/neu FISH testing using a dual-color system, criteria for positivity for HER2/neu amplification are as follows: HER2/neu/CEP17 ratio of 2.0 or greater, or average HER2/neu of at least 6 copies per cell; criteria for negativity are as follows: HER2/neu/CEP17 ratio less than 2.0 with average HER2/neu of fewer than 4.0 copies per cell. All other results are considered equivocal for HER2/neu amplification.

Following the 2013 guidelines, centers have reported increased rates of overall HER2/neu positivity (by ~2%) and of equivocal cases (~4%–5%). The increase in equivocal cases results in delay of definitive diagnoses of HER2/neu status. Most of these cases have increased CEP17 copy numbers (3–4/cell nucleus). Previous studies have suggested that tumors with increased HER2/neu copy number respond to anti-HER2/neu treatment, independent of HER2/neu/CEP ratio and even when HER2/neu copy number is in the equivocal range. The equivocal group defined by the 2013 criteria may be enriched for patients who could benefit from anti-HER2/neu therapy. This is in addition to patients whose condition is shifted from equivocal to positive by 2013 criteria. However, the clinical significance of HER2/neu equivocal cases is uncertain. Long et al reported that there is an increased request for additional testing using alternate chromosome 17 probes and even polymerase chain reaction assays for breast cancers with both IHC 2+ and equivocal FISH results. Additional testing will increase costs but with uncertain clinical benefit. Until further studies demonstrate how HER2/neu equivocal cases respond to therapy, pathologists will be revised as follows: “If the initial HER2 testing result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test may be ordered on the excision specimen.” These revisions are expected to be published shortly.

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Tumor-infiltrating lymphocytes (TILs) are important immunologic biomarkers in breast carcinoma, as in many other cancers. The immune system is constantly engaged in immune surveillance. Immune responses may control nascent tumors and influence patient outcomes. The specific mechanisms involved are still being debated. Chemotherapy and radiotherapy may trigger immunologic memory and help to control residual disease. The role and importance of TILs appear to vary significantly among different subtypes of breast cancer. For example, TILs were found to be a positive prognostic biomarker in TNBC but not in the luminal breast cancers. High levels of TILs were associated with good prognosis in HER2/neu-positive carcinomas treated with chemotherapy and HER2/neu-targeted therapy in an adjuvant setting. Mao et al found that higher numbers of TILs in the pretreatment biopsy specimen correlated with better pathologic complete response in HER2/neu+ and TNBC but not in ER+ breast cancer. While the specific meaning of TILs in any given case is still under scrutiny, it is clear that the analysis of the TIL amount and composition is likely to become an integral part of pathologic evaluation. In fact, for this purpose, an international working group has proposed guidelines for standardized evaluation of TILs in breast cancer for daily clinical and research practice.

Some of the salient points of the consensus guidelines in the evaluation of TILs are as follows: Stromal TILs are the immune cells in the stromal tissue, excluding the tumor cells. Intratumoral TILs are the immune cells within the tumor that are in direct contact with malignant cells. Tertiary lymphoid structures are located in the area surrounding the tumor and are defined as consisting of a T-cell zone next to a B-cell follicle that often has germinal centers. These are difficult to distinguish from lymphoid aggregates and are not recommended for clinical assessments. Stromal TIL assessment has been found to be easier, superior, and more reproducible than intratumoral TIL assessment. Stromal TILs have been found to be predictive of increased response to adjuvant and neoadjuvant therapy. In scoring for TILs, all mononuclear lymphoid cells (including lymphocytes and plasma cells) are counted, but polymorphonuclear leukocytes are excluded. Average TILs in the tumor area should be assessed without focusing on hot spots. Lymphocyte-predominant breast cancer is defined as those cancers with at least 50% to 60% of the stromal area occupied by TILs. Evaluation of TILs in colon cancer is already listed as optional in the CAP cancer protocol checklist. It may be only a matter of time before evaluation of TILs is added to the breast cancer protocol checklist as well.

**THE ROLE OF PATHOLOGISTS IN ASSESSING CASES WITH NEOADJUVANT THERAPY**

Neoadjuvant systemic therapy (ie, therapy rendered before surgery) is now widely used for a significant proportion of breast cancers including locally advanced disease and inflammatory breast cancer, and is also increasingly used for patients with early-stage breast cancer. Pathologic complete response (pCR) is defined as the absence of residual invasive carcinoma in the breast and lymph nodes at the time of surgery and is an excellent prognostic indicator. Thus, pCR was approved by the FDA as a surrogate endpoint for clinical trials and drug approval. The strongest correlation between pCR and outcomes is found in HER2/neu+ cancer and TNBC. Houssami et al reported that pCR occurred in about 9% of hormonal receptor (HR)+/HER2/neu–, 19% of HR+/HER2/neu+, 39% of HR+/HER2/neu+, and 31% of TNBCs. If pCR is not achieved, residual cancer burden in the breast and nodes is associated with increased regional recurrence and decreased survival. Therefore, accurate assessment of pCR or residual cancer burden is crucial.

To ensure a standard approach to evaluate tumor response to neoadjuvant therapies, a multidisciplinary international working group convened by the Breast International Group–North American Breast Cancer Group leadership developed recommendations for the pathologic assessment of residual cancer burden. While these recommendations are intended for clinical trials but not routine practice, it is becoming increasingly used as a part of routine practice in tertiary care institutions. We refer readers to the original publication for detailed recommendations. The key elements for pathologists, which we follow in our institution, are listed below:

1. The specimen is evaluated in the context of pretreatment clinical and imaging findings.
2. Systematic sampling of areas to include the largest cross-section of the fibrotic tumor bed is performed. Up to 5 blocks of the largest cross-section, with a maximum of ~25 blocks of the entire tumor bed is sufficient. Mapping of the specimen and sections taken is strongly recommended.
3. Residual disease may exhibit a “scatter” pattern, with multiple tumor foci in a large tumor bed. It is recommended to document the 2 dimensions of the largest cross-section of tumor bed involved by residual viable invasive carcinoma and average residual tumor cellularity (method A in Figure 2). These are likely a better indicator of therapeutic response, compared to the 7th edition American Joint Commission on Cancer (AJCC) staging system, which considers these as multiple foci and measures the largest focus (method B in Figure 2).
4. Size of largest lymph node metastasis is documented. Lymph nodes with treatment effect only without residual carcinoma are regarded as negative lymph nodes.
5. Residual lymphovascular invasion is documented and is not classified as pCR.
6. Repeated testing of ER, PR, and HER2/neu is not required but may be helpful.

The detailed protocol for breast residual cancer burden evaluation is available at the MD Anderson Cancer Center Web site.

**EVOLVING TECHNOLOGIES WITH IMPLICATIONS FOR PATHOLOGY: RADIOACTIVE SEED LOCALIZATION**

Screening mammography and improved imaging technology have made detection of nonpalpable breast lesions possible, and currently about a third of radiologically suspicious breast lesions are clinically occult. Image-directed localization of nonpalpable breast lesions is necessary to perform breast-conserving procedures.

The most commonly used method for the surgical excision of nonpalpable breast lesions is wire-guided localization (WGL). This method has many inherent problems. The...
entry site of the wire in a nonuniform specimen may result in asymmetric surgical margins,\textsuperscript{123} which could lead to false-positive margins requiring unnecessary re-excision.\textsuperscript{124–126} Disadvantages also include wire transection or displacement,\textsuperscript{127,128} retained metallic fragments, and failed localization due to the “accordion” effect from breast tissue decompression, which could cause removal of excessive tissue or even failure to excise the targeted lesion.\textsuperscript{129,130} In addition, with the need for close coordination among radiologists, operating room staff, and the surgeon for same-day procedures, WGL may represent a logistical challenge.

To overcome some of those challenges, radio-guided localization techniques have been developed. Currently, there are 2 main techniques: radio-guided occult lesion localization and radioactive seed localization (RSL). Radio-guided occult lesion localization uses colloidal human serum albumin particles labeled with radioactive technetium (99mTc) that is inoculated directly into the lesion during mammography or ultrasound procedure.\textsuperscript{131} More recently, RSL was introduced and a pilot study found it to be a safe means of localization with 100% pathologic confirmation of lesion removal.\textsuperscript{124,132,133} Radioactive seed localization is now being used in multiple institutions.

Increased patient comfort is one of many advantages of RSL. Patients with RSL rated it less painful while causing the same level of anxiety as compared with WGL.\textsuperscript{134} Radioactive seed localization saves the patient from being transported between departments with a wire protruding out of the skin, which can dislodge, fracture, or produce pain with movement. Radioactive seed localization can be done ahead of time, greatly simplifying operating room schedules, improving turnaround time, and minimizing patient inconvenience.\textsuperscript{135} Radioactive seed localization may also offer the surgeon continuous real-time acoustic orientation resulting in a more uniform specimen centered around the radioactive seed. The long half-life of the radioactive seed, iodine 125, allows for RSL placement before neoadjuvant chemotherapy. The cost of the procedure was reported to be comparable to that of WGL,\textsuperscript{136} especially if a large volume of cases is performed.\textsuperscript{137,138}

In the RSL procedure, the radioactive seed is introduced with an 18-gauge needle during standard ultrasound or mammographic exam. Sentinel lymph node mapping and dissection is possible during the RSL procedure by using different radioactive materials. The gamma-probe can confirm the presence of the seed in the specimen, which can be further confirmed by specimen radiography. The gamma-probe also helps the pathologist retrieve the seed during intraoperative examination. In our department, the specimen is delivered to the pathology frozen section room where gross examination and inking of surgical margins are performed. The probe helps to identify the area of maximum activity. The seed is retrieved and placed in a dedicated container for return to the radiology department staff. All these steps are carefully documented in the electronic chart. The specimen should be handled with care, as cutting the seed may lead to the theoretical risk of radioactive iodine becoming airborne. However, this is a remote possibility, as iodine 125 is covalently bound in a halide silver wire or an exchange resin encased in a titanium shell.\textsuperscript{125}

Performance of these procedures requires proper licensing, which is rigorous.\textsuperscript{123} The RSL licensing program requires routine and emergency training of all persons involved in handling the radioactive seeds. The receipt, storage, implantation by radiologists, surgical excision by surgeons, retrieval by pathologists, and return of seeds to the radiology department and decay of the seeds should be carefully documented according to the protocol developed by each institution.\textsuperscript{135} Failure to do so may result in temporary suspension of RSL procedures until a corrective protocol is put in place.\textsuperscript{138}

References
1. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature. 2000;406(6797):747–752.
2. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A. 2001;98(19):10664–10674.
3. O’Brien KM, Cole SR, Tse CK, et al. Intrinsic breast tumor subtypes, race, and long-term survival in the Carolina Breast Cancer Study. Clin Cancer Res. 2010;16(24):6100–6110.
4. 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(10):736–750.
5. Cheang MC, Voduc D, Djabli C, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res. 2008;14(5):1368–1376.
6. Kim MJ, Ro JY, Ahn SH, Kim HH, Kim SB, Gong G. Clinicopathologic significance of the basal-like subtype of breast cancer: a comparison with hormone receptor and Her2/neu-overexpressing phenotypes. Hum Pathol. 2006;37(9):1217–1226.
7. Kreike B, van Kooijhove M, Horlings H, et al. Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. Breast Cancer Res. 2007;9(5):R65.
in triple-negative breast cancers. 

8. Rao C, Shetty J, Prasad KH. Immunohistochemical profile and morphology in triple-negative breast cancers. J Clin Diagn Res. 2013;7(1):1361–1365.

9. Livasy CA, Karaca G, Nanda R, et al. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol. 2006;19(2):264–271.

10. Blows FM, Driver KE, Schmidt MK, et al. Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. PLoS Med. 2010;7(5):e1000279.

11. Martinez AP, Cohen H, Hanley KZ, Li XB. Estrogen receptor and cytokeratin 5-4 expression in triple-negative cancer cells. J Histochem Cytochem. 2009;57(4):249–252. 2009;295(6871):530–536.

12. Leu D, Eckhardt BL, Lim B, et al. The prevalence of intrinsic subtypes and prognosis in breast cancer patients of different races. Breast. 2007;16(suppl 2):S72–S77.

13. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and surgical management. J Natl Cancer Inst. 2008;100(9):672–679.

14. Weigelt B, Wierzbicki JK, Moore J, et al. Metaplastic breast carcinomas display a naturally occurring breast cancer subset enriched in epithelial-to-mesenchymal transition and stem cell characteristics. Cancer Res. 2009;69(10): 4116–4124.

15. Haakensen VD, Lingjaerde OC, Luders T, et al. Gene expression profiles of breast biopsy specimens from healthy women identify a group with claudin-low breast cancer. BMC Med. 2011;9:41.

16. Liedtke C, Mazouni C, Hess KR, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol. 2008;26(12):1275–1281.

17. Gonzalez-Angulo AM, Timms KM, Liu S, et al. Incidence and outcome of BRCA mutations in unselected patients with triple-negative breast cancer. Cancer Cell. 2011;17(5):1082–1089.

18. van’t Veer LJ, Dai H, van de Vijver MJ, et al. Gene expression profiling predicts clinical outcome of breast cancer. Nature. 2002;415(6871):530–536.

19. Lygren R, Bergh J, de Thonel E, et al. Immunohistochemistry to investigate a relationship between subtype and short term survival in triple receptor-negative breast cancer of the breast appears more aggressive than other triple receptor-negative breast cancers. Breast. 2012;21(5):580–591.

20. Marty B, Maire V, Gravier E, et al. Frequent PTEN genomic alterations and activated phosphatidylinositol 3-kinase kinase in basal-like breast cancer cells. Breast Cancer Res. 2008;10(6):R100.

21. Martineau AP, Cohen H, Hanley KZ, Li XB. Estrogen receptor and cytokeratin 5-4 expression in triple-negative cancer cells. J Histochem Cytochem. 2009;57(4):249–252. 2009;295(6871):530–536.

22. Haakensen VD, Lingjaerde OC, Luders T, et al. Gene expression profiles of breast biopsy specimens from healthy women identify a group with claudin-low breast cancer. BMC Med. 2011;9:41.

23. Creighton CJ, Li X, Landis M, et al. Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. Proc Natl Acad Sci U S A. 2009;106(13):18320–18325.

24. Leibman BD, Baez EA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest. 2011;121(7):2750–2767.

25. Hinessey BT, Gonzalez-Angulo AM, Stemke-Hale K, et al. Characterization of a naturally occurring breast cancer subset enriched in epithelial-to-mesenchymal transition and stem cell characteristics. Cancer Res. 2009;69(10): 4116–4124.

26. Le X, Lewis MT, Huang J, et al. Increased resistance of tumorigenic breast cancer cells to chemotherapy. J Natl Cancer Inst. 2008;100(9):672–679.

27. Hennessy BT, Guardano S, Broglio K, et al. Biphasic metastatic sarcomatoid cancer of the breast. Ann Surg Oncol. 2010;17(5):605–613.

28. Lester TR, Hunt KK, Nayeemuddin KM, et al. Metaplastic sarcomatoid carcinoma: a strategy to treat cancer. Expert Opin Pharmacother. 2015;16(18):2751–2758.

29. van der Poll R, Marchetti S, Steeghs N, et al. Long-term survival and anti-tumor activity of olaparib monotherapy after combination with carboplatin and paclitaxel. Br J Cancer. 2016;115(3):396–402.

30. von Minckwitz G, Martin M. Neoadjuvant treatments for triple-negative breast cancer (TNBC). Ann Oncol. 2012;23(suppl 2):v315–v339.

31. Lehmann BD, Pieters JPA. Clinical implications of molecular heterogeneity in triple negative breast cancer. Breast. 2015;24(suppl 2):S36–S40.

32. Masuda H, Baggerly KA, Wang Y, et al. Differential response to neoadjuvant chemotherapy among T triple-negative breast cancer molecular subtypes. Clin Cancer Res. 2013;19(19):5533–5540.

33. Shah SP, Roth A, Goya R, et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature. 2012;486(7403):395–399.

34. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490(7418):61–70.

35. Turner N, Lambros MB, Horlings HM, et al. Integrative molecular profiling of triple negative breast cancers identifies alpaca oncogen drivers and potential therapeutic targets. Kardiovaskultets Tidskrift. 2010;129(14):203–213.

36. Fruman DA, Rommel C. PI3K and cancer: lessons, challenges and opportunities. Nat Rev Drug Discov. 2014;13(12):140–156.

37. Gewinner C, Wang ZC, Richardson A, et al. Evidence that inositol 1,4,5-trisphosphate 4-phosphatase type II is a tumor suppressor that inhibits PI3K signaling. Cancer Cell. 2009;16(2):115–125.

38. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004;304(5667):554–555.
differentially or dedifferentiated liposarcoma: an exploratory proof-of-mechanism study. Lancet Oncol. 2012;13(11):1133–1140.

6. Leijen S, Beijnen JH, Schellens JH. Abrogation of the G2 checkpoint by inhibition of Wee-1 kinase results in sensitization of p35-deficient tumor cells to DNA-damaging agents. Curr Clin Pharmacol. 2010;5(3):186–191.

7. Baserga J, Norton L, Masui H, et al. Antitumor effects of doxorubicin in combination with anti-epidermal growth factor receptor monoclonal antibodies. J Natl Canc Inst. 1993;85(16):1327–1333.

8. Baserga J, Gomez F, Greil R, et al. Randomized phase II study of the anti-epidermal growth factor receptor monoclonal antibody cetuximab with cisplatin and in vivo. Clin Cancer Res. 2011;17(16):5273–5279.

9. Kuznetsov A, McBurney T, Walther EJ. Molecular mediators of tumor angiogenesis: enhanced expression and activation of vascular endothelial growth factor receptor KDR in primary breast cancer. Int J Cancer. 2013;132(10):2358–2368.

10. McDermott J, Jimeno A. Pembrolizumab: PD-1 inhibition as a therapeutic strategy in cancer. Drugs Today (Barc). 2015;51(1):7–20.

11. Ali HR, Glent SE, Blows FM, et al. PD-L1 protein expression in breast cancer is rare, enriched in basal-like tumours and associated with infiltrating lymphocytes. Ann Oncol. 2015;26(7):1488–1493.

12. Alsalman A, Colak D, Al-Harazi O, et al. Bidirectional crosstalk between PD-L1 expression and epithelial to mesenchymal transition: significance in chemoresistant breast cancer. Mol Cancer. 2014;13:149.

13. Mottendorf EA, Philips AV, Meric-Bernstam F, et al. PD-L1 expression in triple-negative breast cancer. Cancer Immunol Res. 2014;2(4):361–370.

14. Muenst E, Schafer AI, Gao F, et al. Expression of programmed death ligand 1 (PD-L1) is associated with poor prognosis in human breast cancer. Breast Cancer Res Treat. 2014;143(1):65–74.

15. Qin T, Zeng YD, Qin G, et al. High PD-L1 expression was associated with poor prognosis in 870 Chinese patients with breast cancer. Oncotarget. 2015;6;32(37):90814.

16. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med. 2007;131(11):18–43.

17. Wolter RW, Hammert AM, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. Arch Pathol Lab Med. 2014;138(2):241–256.

18. Keating GM. Pertuzumab: in the first-line treatment of HER2-positive breast cancer. Drugs. 2012;72(3):353–360.

19. Rakha EA, Picher M, Shaaban A, et al. National guidelines and level of evidence: comments on some of the new recommendations in the American Society of Clinical Oncology and the College of American Pathologists human epidermal growth factor receptor 2 guidelines for breast cancer. J Clin Oncol. 2015;33(11):1301–1302.

20. Rakha EA, Starczynski J, Lee AH, Ellis IO. The updated ASCO/CAP HER2 guideline recommendations for HER2 testing in the management of invasive breast cancer: a critical review of their implications for routine practice. Histopathology. 2014;64(4):609–615.

21. Hammond ME, Hicks DG. American Society of Clinical Oncology/College of American Pathologists human epidermal growth factor receptor 2 testing: clinical practice guideline update: methods: proof that clinical practice guidelines are living documents. Arch Pathol Lab Med. 2015;139(8):970–971.

22. Lee AH, Key HP, Bell JA, Hodi F, Ellis IO. Concordance of HER2 status assessed on needle core biopsy and surgical specimens of invasive carcinoma of the breast. Histopathology. 2012;60(6):880–884.

23. Lebeau A, Turzyński A, Braun S, et al. Reliability of human epidermal growth factor receptor 2 immunohistochemistry in breast core needle biopsies. J Clin Pathol. 2010;63(12):1270–1272.

24. Chen X, Yuan Y, Gu Z, Shen K. Accuracy of estrogen receptor, progesterone receptor, and HER2 status between core needle and open excision biopsy in breast cancer: a meta-analysis. Breast Cancer Res Treat. 2012;134(3):955–966.

25. Rakha EA, El-Sayed ME, Lee AH, et al. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. J Clin Oncol. 2008;26(19):3153–3158.

26. Muller KE, Marinoti JD, Memoli VA, Wells WA, Tafe LJ. Impact of the 2013 ASCO/CAP HER2 guideline updates at an academic medical center that performs primary HER2 FISH testing: increase in equivocal results and utility of reflex immunohistochemistry. Am J Clin Pathol. 2013;144(2):247–252.

27. Long TH, Lawe H, Durum C, et al. The new equivocal: changes to HER2 FISH testing guidelines after applying the 2013 ASCO/CAP guidelines. Am J Clin Pathol. 2014;142(2):253–262.

28. Varga Z, Nöske A. Impact of modified 2013 ASCO/CAP guidelines on HER2 testing in breast cancer: one year experience. PloS One. 2015;10(10):e0140652.

29. Swanson PE, Yang H. Is “polysomy” in breast carcinoma the “new equivocal” in HER2 testing? Am J Clin Pathol. 2015;144(2):181–184.

30. Hofmann M, Stoss O, Gaiser T, et al. Central HER2 IHC and FISH analysis in a trastuzumab (Herceptin) phase II monotherapy study: assessment of test sensitivity and impact of chromosome 17 polysomy. PloS Pathog. 2008;4(11):e99–94.

31. Hanna WM, Ruschho J, Bilous M, et al. HER2 in situ hybridization in breast cancer: clinical implications of polysomy 17 and genetic heterogeneity. Appl Pathol. 2014;32(7):1–16.

32. Demaria S, Pikarsky E, Karin M, et al. Cancer and inflammation: promise for biologic therapy. J Immunother. 2010;33(4):335–351.

33. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006;313(5784):1966–1964.

34. Mahmoud KM, Freeman GJ, McDermott DF. The next immune-checkpoint inhibitors: PD-1/PD-L1 blockade in melanoma.

35. Lepoutre M, Brouard C, Yatabe Y, et al. Human epidermal growth factor receptor 2 immunohistochemistry in breast core needle biopsies: a critical review of the implications for routine practice. J Clin Oncol. 2015;33(4):2770–2775.
chemotherapy correlates with breast cancer outcome. *Ann Surg Oncol*. 2003;10(7):714–719.

121. Sajid MS, Parampalli U, Haider Z, Bonomi R. Comparison of radioguided occult lesion localization (ROLL) and wire localization for non-palpable breast cancers: a meta-analysis. *J Surg Oncol*. 2012;105(8):852–858.

122. Lovrics PJ, Comacchi SD, Farrokhyar F, et al. The relationship between surgical factors and margin status after breast-conservation surgery for early stage breast cancer. *Am J Surg*. 2009;197(6):740–746.

123. Jakub JW, Gray RJ, Degnim AC, Boughhey JC, Gardner M, Cox CE. Current status of radioactive seed for localization of non palpable breast lesions. *Am J Surg*. 2010;199(4):522–528.

124. Gray RJ, Salud C, Nguyen K, et al. Randomized prospective evaluation of a novel technique for biopsy or lumpectomy of nonpalpable breast lesions: radioactive seed versus wire localization. *Ann Surg Oncol*. 2001;8(9):711–715.

125. Intra M, de Cicco C, Gentilini O, Luini A, Paganelli G. Radioguided localisation (ROLL) of non-palpable breast lesions and simultaneous sentinel lymph node biopsy (SNOLL): the experience of the European Institute of Oncology. *Eur J Nucl Med Mol Imaging*. 2007;34(6):957–958.

126. Stock RG, Stone NN. Permanent radioactive seed implantation in the treatment of prostate cancer. *Hematol Oncol Clin North Am*. 1999;13(3):489–501.

127. Montrey JS, Levy JA, Brenner RJ. Wire fragments after needle localization. *AJR Am J Roentgenol*. 1996;167(5):1267–1269.

128. Homer MJ. Transection of the localization hooked wire during breast biopsy. *AJR Am J Roentgenol*. 1983;141(5):929–930.

129. Homer MJ, Pile-Spellman ER. Needle localization of occult breast lesions with a curved-end retractable wire: technique and pitfalls. *Radiology*. 1986;161(2):547–548.

130. Ernst MF, Avenarius JK, Schuur KH, Roukema JA. Wire localization of non-palpable breast lesions: out of date? *Breast*. 2002;11(5):408–413.

131. Luini A, Zurrida S, Galimberti V, Paganelli G. Radioguided surgery of occult breast lesions. *Eur J Cancer*. 1998;34(1):204–205.

132. Pouw B, de Wit-van der Veen LJ, Stokkel MP, Loo CE, Vrancken Peeters MJ, Valdes Olmos RA. Heading toward radioactive seed localization in non-palpable breast cancer surgery: a meta-analysis. *J Surg Oncol*. 2015;111(2):185–191.

133. van Riet YE, Jansen FH, van Beek M, van de Velde CJ, Rutten HJ, Nieuwenhuijzen GA. Localization of non-palpable breast cancer using a radiolabelled titanium seed. *Br J Surg*. 2010;97(8):1240–1245.

134. Lovrics PJ, Goldsmith CH, Hodgson N, et al. A multicentered, randomized, controlled trial comparing radioguided seed localization to standard wire localization for nonpalpable, invasive and in situ breast carcinomas. *Ann Surg Oncol*. 2011;18(12):3407–3414.

135. Murphy JO, Moo TA, King TA, et al. Radioactive seed localization compared to wire localization in breast-conserving surgery: initial 6-month experience. *Ann Surg Oncol*. 2013;20(13):4121–4127.

136. Cox CE, Furman B, Stowell N, et al. Radioactive seed localization breast biopsy and lumpectomy: can specimen radiographs be eliminated? *Ann Surg Oncol*. 2003;10(9):1039–1047.

137. McGhan LJ, McKeever SC, Pockaj BA, et al. Radioactive seed localization for nonpalpable breast lesions: review of 1,000 consecutive procedures at a single institution. *Ann Surg Oncol*. 2011;18(11):3096–3101.

138. Rao R, Moldrem A, Sarode V, et al. Experience with seed localization for nonpalpable breast lesions in a public health care system. *Ann Surg Oncol*. 2010;17(12):3241–3246.