CME/SAM

Triple-Negative Breast Carcinoma

Current and Emerging Concepts

Robert Schmadeka, MD, Bryan E. Harmon, MD, and Meenakshi Singh, MD

From the Department of Pathology, Stony Brook University School of Medicine, State University of New York at Stony Brook, Stony Brook, NY.

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ABSTRACT

Objectives: *Triple-negative breast cancer is regarded as an aggressive disease that affects a young patient population and for which effective targeted therapy is not yet available.*

Methods: *Intense efforts have been made to gain a better understanding of this heterogeneous group of tumors from the histologic to the genomic and molecular levels.*

Results: *Progress has been made, including the ability to subtype these tumors and the discovery of biomarkers toward which current therapeutic efforts are focused. Many novel targets under exploration have the potential to affect the clinical course of this disease.*

Conclusions *This article reviews the current concepts regarding the clinicopathologic features of triple-negative breast carcinoma, its histologic subtypes, molecular classification, the prognostic and therapeutic potential of biomarkers, and emerging targeted therapies.*

Upon completion of this activity you will be able to:

- list the histologic features that suggest a triple-negative immunophenotype.
- compare the features of triple-negative breast cancers and basal-like breast cancers.
- discuss the relationship between the triple-negative phenotype, the basal-like phenotype, and breast cancers with the *BRCA1* mutation.
- define biomarkers that have been identified as potential therapeutic targets in triple-negative breast cancer and apply why these particular biomarkers represent promising targets.

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Breast cancer is the most common cancer among women in the United States and the second most common cause of cancer mortality in US women. In 2012, an estimated 226,870 US women were diagnosed with breast cancer.[1] Approximately 12% to 24% of these cases are categorized as triple-negative breast cancer (TNBC).[2] TNBC is defined immunohistochemically as breast cancer that does not overexpress human epidermal growth factor receptor 2 (HER2, ERBB2) and is estrogen receptor (ER) and progesterone receptor (PR) negative. Although defined immunohistochemically, TNBCs often have radiologic and morphologic features that distinguish them from non-TNBCs. With the current lack of effective targeted therapies for TNBC, treatment regimens often fail to slow tumor progression. Accurate identification

of TNBCs and adequately powered prospective trials are necessary to validate predictive biomarkers and to establish effective treatments. Our aim is to review the literature on TNBC, including its clinicopathologic features, histologic subtypes, molecular classification, biomarkers and their prognostic and therapeutic potential, and emerging treatment options.

Clinical Features

Compared with non-TNBCs, a larger proportion of TNBCs occur in younger women, particularly of African American or West African ancestry, and in women of low socioeconomic status.[3] Newly diagnosed breast carcinomas in African American women are twice as likely to be triple negative than those diagnosed in white women.[4] It has been suggested that Hispanic women are more likely to present with TNBC than white women[5]; however, other studies have not found this difference to be significant.[6]

TNBCs tend to behave more aggressively than non-TNBCs. Patients with TNBC tend to experience a relapse more quickly and have a higher likelihood of developing central nervous system and visceral metastases than those with non-TNBC.[7] In a study of 1,601 women with breast cancer, 180 women with TNBC had a distant recurrence rate of 33.9% compared with 20.4% among women with non-TNBCs.[8]

Imaging

Radiology-pathology correlations are integral to the diagnosis and therapy planning for breast cancer. The most common presentation of TNBC on mammography is that of a mass without associated calcifications (49%-100% of cases).[9] Common mammographic features of TNBC include a hyperdense mass (89.3%) with oval (68.9%) or lobular shape (28.6%) and indistinct (42.9%) or circumscribed margins (32.1%).[10] Features typical of non-TNBCs, including irregular shape, spiculated margins, and associated suspicious calcifications, are less frequently present in TNBCs.[9] Despite their large size, TNBCs may be occult on mammography (18% of cases). When screening patients who are at an increased risk for TNBC, it may be beneficial to include other imaging modalities such as ultrasonography or magnetic resonance imaging (MRI).

Ultrasonography has shown 92% to 100% sensitivity for the detection of TNBC.[9] On ultrasonography, TNBCs present as a distinct mass with circumscribed margins in 21% to 27% of cases. Posterior acoustic enhancement is seen in 24% to 41% of cases and can be indicative of an internal fluid component, such as necrosis, a common feature of TNBCs. However, circumscribed margins and posterior acoustic enhancement are also commonly found in benign neoplasms, cysts, and abscesses. TNBCs are usually irregular (68.9%) or oval (28.9%) in shape with microlobulated (46.7%), circumscribed (17.8%), or indistinct margins (17.8%).[10]

MRI has been reported to yield the highest sensitivity for TNBC.[9] Rim enhancement on MRI is considered highly predictive of malignancy, and this is a common feature of TNBCs (present in 76% of cases).[9] Additional MRI features described for TNBC include mass enhancement, areas of high intratumoral T2 signal intensity, lobulated shape, and smooth margins.[11] Despite the increased sensitivity of both ultrasonography and MRI in detecting breast cancers, both are associated with a high false-positive rate.[12]

Mammography, ultrasonography, and MRI in conjunction provide the greatest sensitivity for detecting breast cancers, but this strategy results in an increased number of false-positive findings and a decreased positive predictive value.[13] **Image 1** shows mammographic (A), ultrasonographic (B), and MRI (C) studies of one example of TNBC. Unfortunately, most of these lesions are not detected early via imaging. Most TNBCs are detected either as a palpable mass or because the patient has symptoms of breast pain or nipple discharge.[14]

Pathologic Features

Histologically, most TNBCs are classified as high-grade invasive ductal carcinoma, no specific type (Image 1, D).[15] TNBCs are not defined by their appearance on H&E stain but by their lack of expression of ER, PR, and HER2 on immunohistochemical staining (Image 1E, Image 1F, and Image 1G). However, there are histologic features that suggest a triple-negative immunophenotype. TNBCs are typically characterized by a high histologic grade, with central necrotic zones and pushing borders[16,17] **Image 2A** and **Image 2B**. Triple-negative tumors often demonstrate a cellular fibrous proliferation **Image 2C** and **Image 2D**, whereas non-TNBCs tend to have a fibrosis with a greater degree of hyalinization. Variably sized blood vessels, including thick-walled vessels, are frequently found in TNBCs **Image 2E** and **Image 2F**. These histologic features may correlate with the biomarkers and therapeutic targets that are discussed later in this review. A study in a strictly Asian population showed an older age at diagnosis with a greater tendency toward having an infiltrative border,[18] which suggests that regional or racial variability may be seen in the clinical presentation and histologic features of TNBC.

Additional features commonly observed in TNBCs are a perilobular lymphocytic infiltrate in breast tissue adjacent to the tumor and an intratumoral lymphocytic inflammatory

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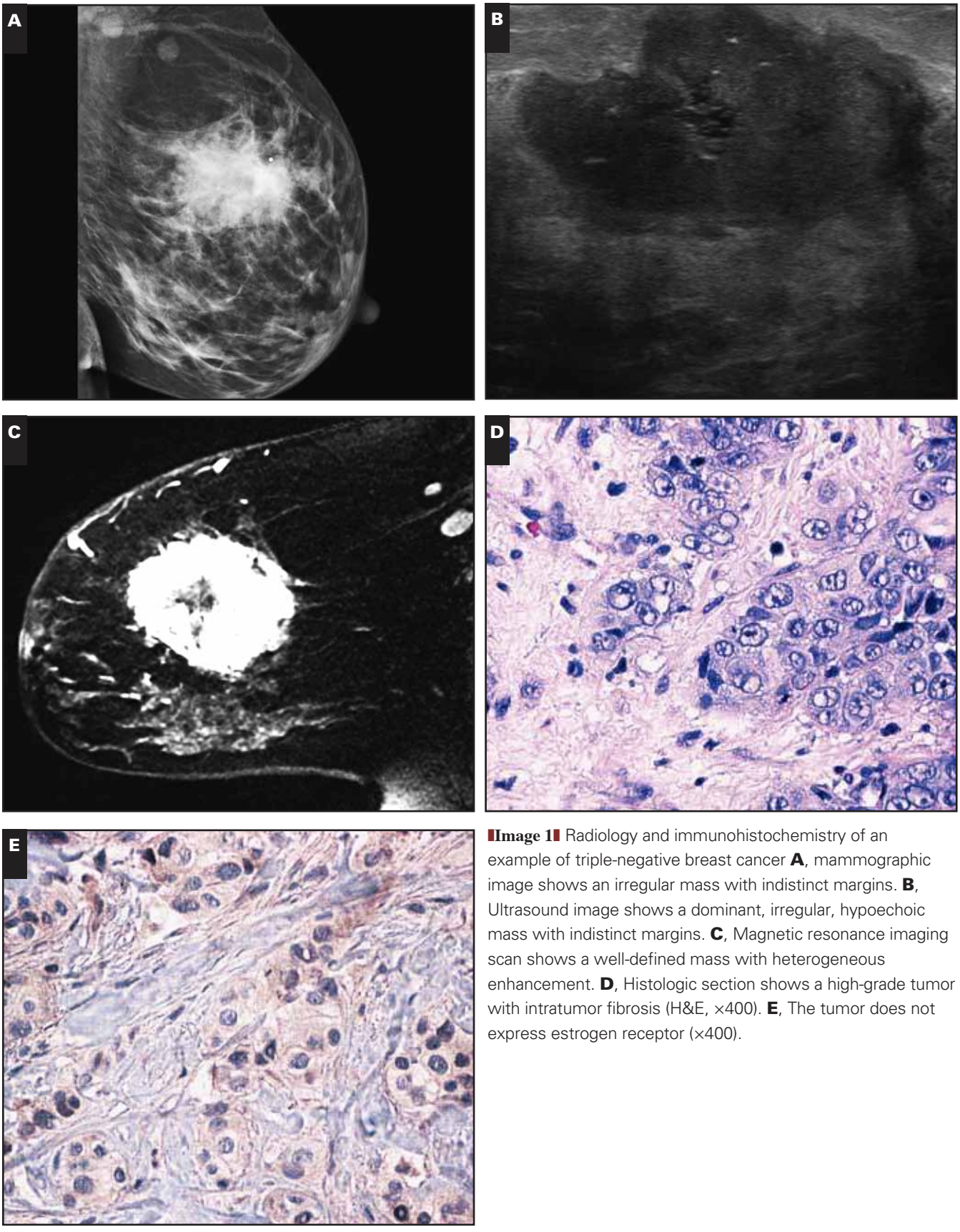

Image 1 Radiology and immunohistochemistry of an example of triple-negative breast cancer **A**, mammographic image shows an irregular mass with indistinct margins. **B**, Ultrasound image shows a dominant, irregular, hypoechoic mass with indistinct margins. **C**, Magnetic resonance imaging scan shows a well-defined mass with heterogeneous enhancement. **D**, Histologic section shows a high-grade tumor with intratumor fibrosis (H&E, ×400). **E**, The tumor does not express estrogen receptor (×400).

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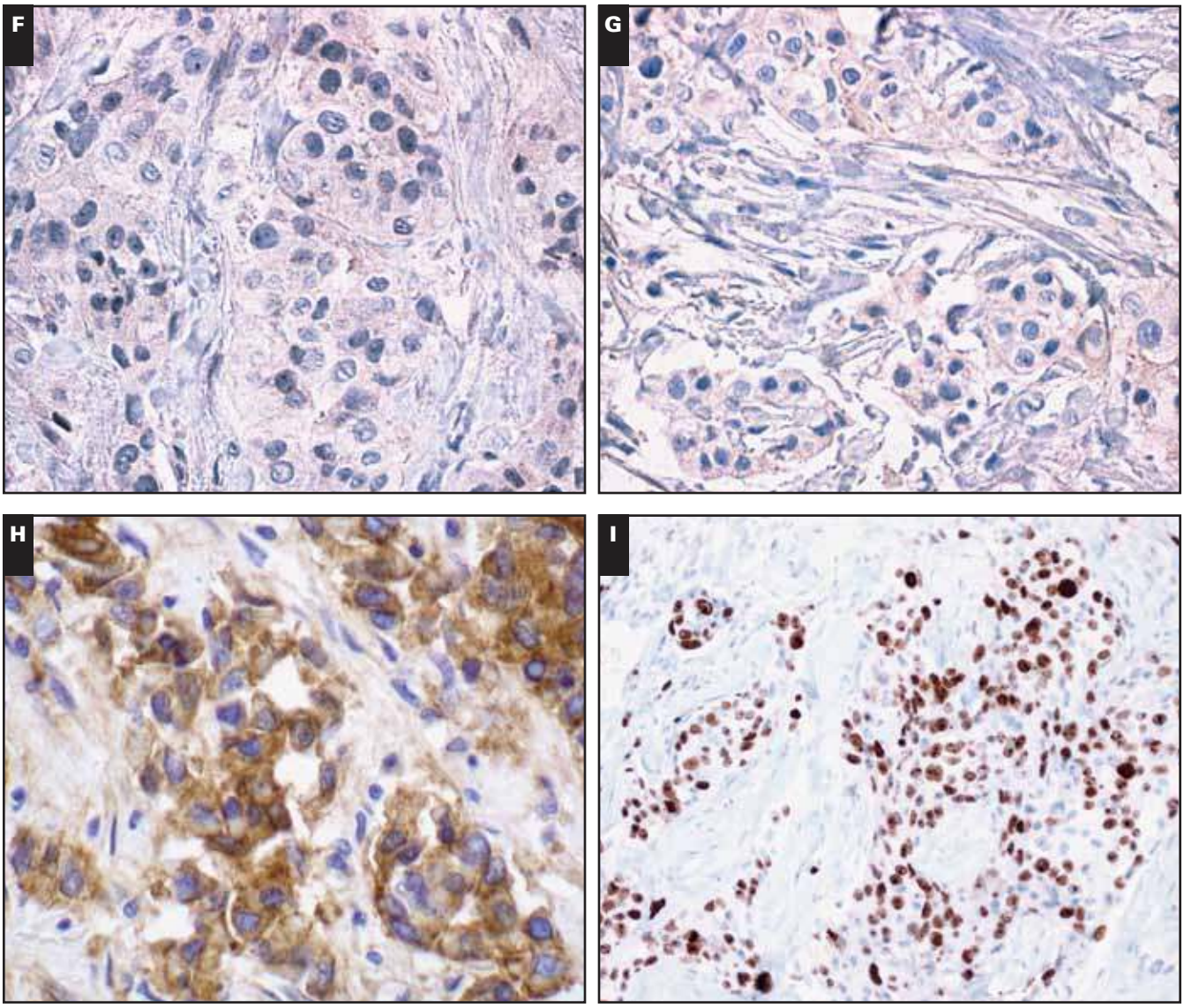

Image 1 (cont) **F**, The tumor does not express progesterone receptor (×400). **G**, The tumor does not overexpress HER2 (×400). **H**, The tumor shows expression of cytokeratin 5 and 6 (×400). **I**, The tumor shows a high proliferative rate with 60% of tumor cell nuclei highlighted by a Ki-67 immunostain (×200).

infiltrate **Image 2 G** and **Image 2H**. Recent studies have demonstrated that B and T lymphocytes can support tumor activity indirectly by regulating the activity of myeloid cells, including macrophages, monocytes, and mast cells.[19] Analogous to the differentiation of helper T cells into type 1 (Th1) or type 2 (Th2) helper T cells, macrophages undergo polarization into two different states (M1 and M2).[20] M1 macrophages induce a Th1 response. M2 macrophages suppress Th1 activity, promote invasion and migration of tumor cells, and promote angiogenesis. Dense infiltration of tumor stroma by M2 macrophages positively correlates with TNBC/basal-like breast cancer and inversely correlates with luminal A breast cancer.[21] The relationship between this finding and the higher recurrence rate of TNBC is an area of ongoing research.

Although the aforementioned histologic features are common in TNBC, these tumors can display various histologic appearances **Image 3**. In addition to intertumor heterogeneity, histologic heterogeneity is often seen within an individual tumor (Image 3B, Image 3C, and Image 3D). This can pose a therapeutic challenge and a diagnostic dilemma when evaluating metastatic lesions if the primary tumor has not been sampled well and thoroughly characterized histologically at the time of initial diagnosis.

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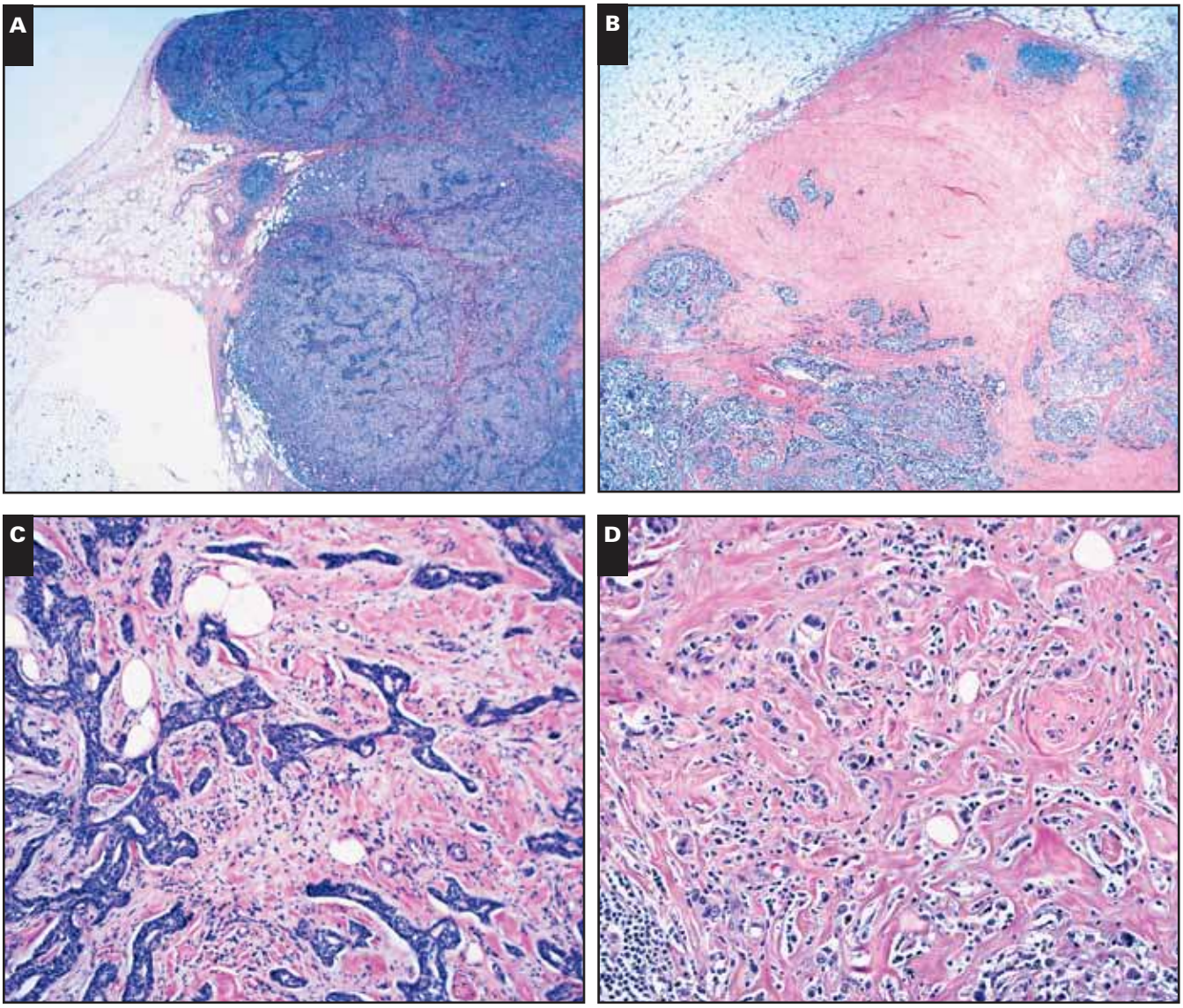

Image 2 Histologic features of triple-negative breast cancer (TNBC). **A** and **B**, TNBC demonstrating a pushing border (H&E, ×20). **C** and **D**, TNBC demonstrating cellular fibrosis (H&E, ×200).

TNBC, Basal-like Breast Carcinoma, and Breast Carcinoma in Patients With *BRCA1* Mutation

The term *basal-like* breast cancer was coined to describe tumors that overexpress genes that characterize breast basal epithelial cells based on microarray gene expression assays. Although the histologic and immunohistochemical phenotypes of TNBCs and basal-like breast cancers overlap, "triple-negative" and "basal-like" are not synonymous terms. A discordance of up to 30% has been described between the two groups. However, because microarray gene expression assays are used mainly in the research setting, clinicians often use the triple-negative definition as a surrogate for basal-like breast cancer.[22]

Basal-like breast cancer comprises 15% to 20% of all breast cancers and, like TNBC, tends to occur in younger premenopausal women of African American and West African descent. Basal-like cancers generally have a poor prognosis.[23] The tumors tend to carry tumor protein p53 (*TP53*) mutations. Histologically, they share features with TNBC. They are high-grade tumors with pushing borders and a stromal lymphocytic response. However, only 55% to 85% of basal-like carcinomas are triple negative on immunohistochemistry. Biomarkers expressed by basal-like breast cancers include cytokeratin (CK) 5/6 (Image 1H), CK14, CK17, laminin, epidermal growth factor receptor (EGFR), fatty acid binding protein, p16, and p53.[2,24,25]

More than 75% of tumors arising in women carrying a germline mutation in *BRCA1* have a triple-negative

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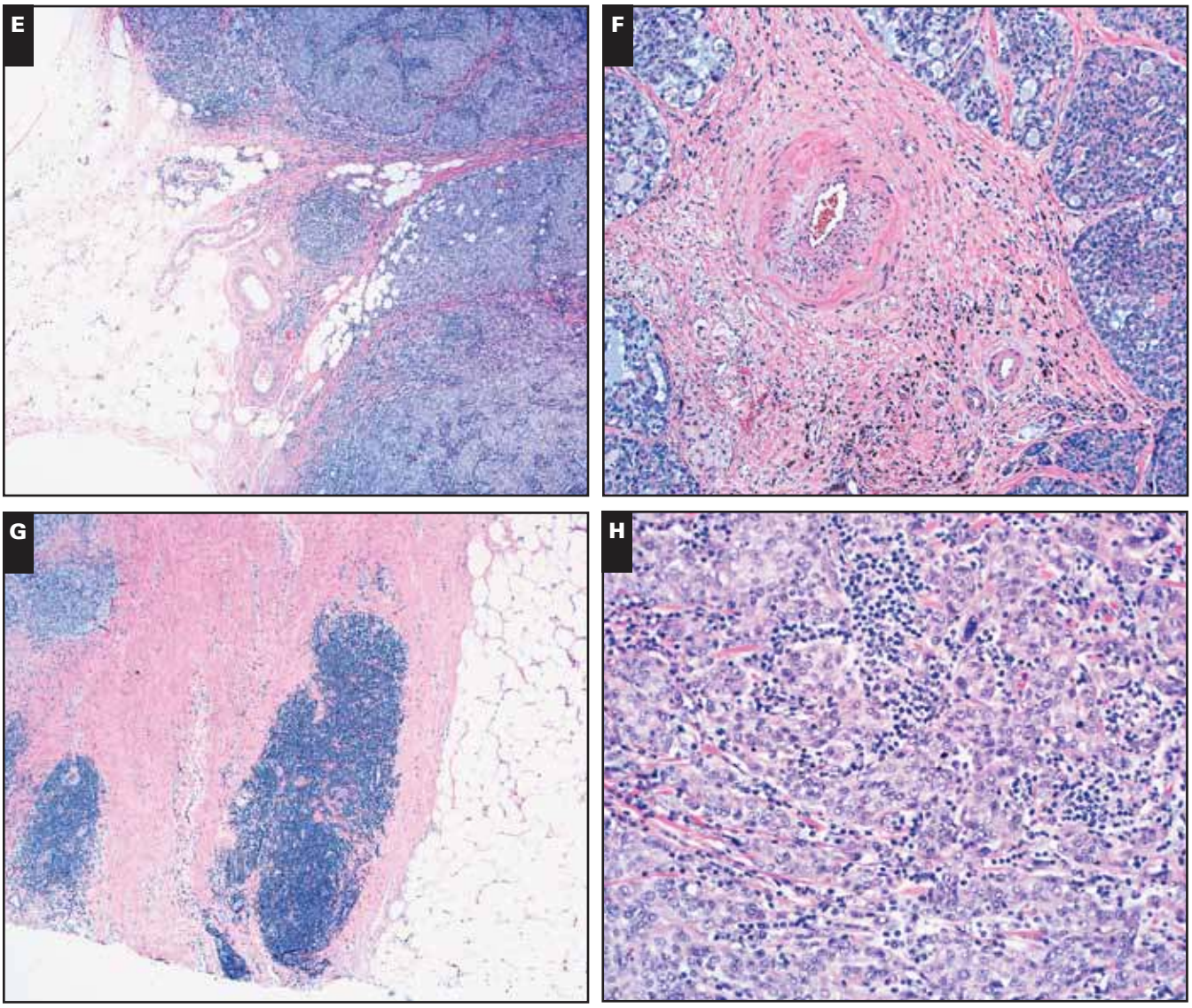

Image 2 (cont) **E** and **F**, TNBC demonstrating neovascularization with thick-walled vessels (H&E, ×40 [**E**] and ×100 [**F**]) **G**, TNBC demonstrating a peritumor lymphocytic infiltrate (H&E, ×40). **H**, TNBC demonstrating intratumoral lymphocytic infiltrate (H&E, ×400).

phenotype, a basal-like phenotype, or both.[26] Mutations in *BRCA1* and *BRCA2* are characterized by defects in the process of homologous recombination in double-stranded DNA break repair. Compared with the general population, patients with germline mutations in either of these genes have a 20- to 30-fold increased risk for breast cancer.[27] Although the proteins encoded by the two genes have different binding substrates, they are part of a common pathway. Despite their common pathway, only mutations in *BRCA1* have an association with basal-like carcinomas.[28]

Special Histologic Subtypes of TNBC

Metaplastic breast carcinoma is a rare entity, comprising less than 1% of all breast carcinomas.[29] It is most often triple negative on immunophenotyping, and genomic profiling places them with the basal-like breast carcinomas.[30] This type of tumor is histologically heterogeneous and can be entirely epithelial in nature or mixed epithelial and mesenchymal. These tumors can be purely metaplastic in composition or can be admixed with other types of invasive carcinoma. Components seen in these tumors that are not typically seen in other breast carcinomas include squamous differentiation, a spindle cell component, and chondroid (Image 3E and Image 3F), osseous, and rhabdomyoid elements.[31] These carcinomas often express EGFR and have a poor prognosis.[29] In patients with lymph node metastasis who underwent adjuvant chemotherapy, the 3-year disease-free survival rate was 44.4% in metaplastic breast carcinomas and 72.5% in triple-negative invasive ductal carcinomas.[32]

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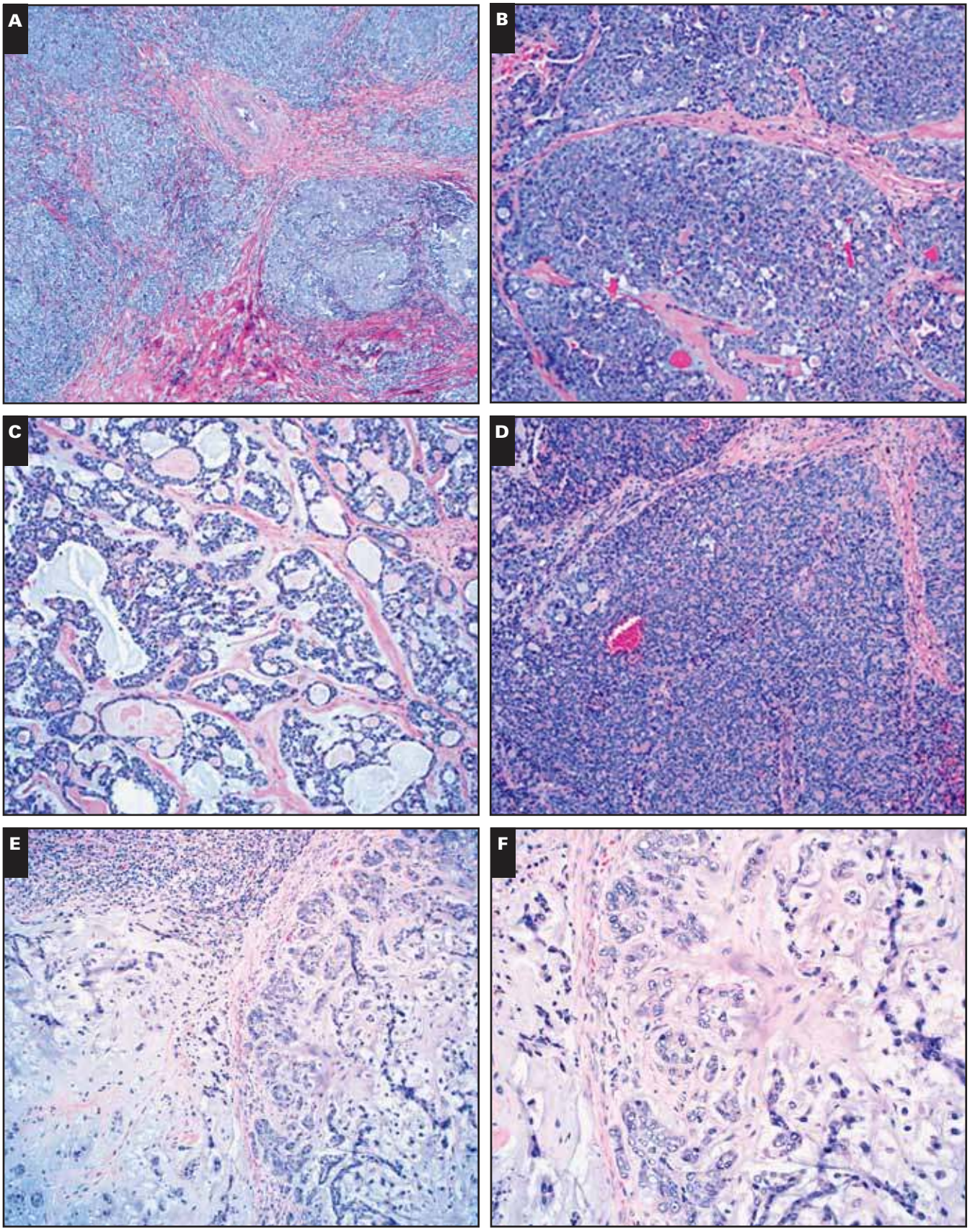

Image 3 Histologic subtypes of triple-negative breast cancer (TNBC). **A**, Invasive ductal carcinoma, high-grade; no in situ component identified (H&E, ×40). **B-D**, Histologic heterogeneity within a TNBC. **B**, Invasive ductal carcinoma component (H&E, ×100). **C**, Adenoid cystic carcinoma component (H&E, ×200). **D**, Neuroendocrine carcinoma component with rosettes (H&E, ×100). **E** and **F**, Metaplastic carcinoma component with chondroid differentiation (H&E, ×100 [**E**] and ×200 [**F**]).

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Medullary carcinomas account for 3% to 5% of invasive breast carcinomas.[33] Histologically, medullary carcinomas are characterized by a prominent syncytial growth pattern, well-circumscribed margins, nuclear pleomorphism, a high mitotic rate, a diffuse lymphoid infiltrate, and the absence of glandular features or an in situ component (Image 3A).[34] These characteristics are often immunophenotypically triple negative. From a molecular standpoint, these carcinomas fall in the basal-like group. Based on some reports, they are best considered a distinct subgroup within the basal-like category.[35] Despite their high-grade histologic features, medullary carcinomas have a better prognosis than invasive breast carcinomas of no special type.

Salivary gland–type neoplasms are uncommon in the breast and represent approximately 2% of primary breast carcinomas. They are characteristically triple negative on immunohistochemistry.[36] Most of these tumors are capable of both epithelial and myoepithelial differentiation, and the amounts of each component vary from case to case. They are analogous to the types of neoplasms that occur in the salivary gland but with some important differences.[37] For example, in the breast, a distinction is made between benign adenomyoepithelioma and malignant adenomyoepithelioma. Epithelial-myoepithelial carcinoma is synonymous with an adenomyoepithelioma in which both components are malignant.[38] In the salivary gland, a distinction between benign and malignant adenomyoepithelioma is not made (all lesions are designated as adenomyoepithelioma),[37] and epithelial-myoepithelial carcinoma is synonymous with adenomyoepithelioma.[39] Adenoid cystic carcinoma of the breast is uncommon and not well characterized. Immunohistochemically, in addition to being triple negative, adenoid cystic carcinoma cells express tumor protein p63 (TP63) and c-KIT. There is no overexpression of TP53. Genetic studies have not revealed mutations in the *TP53* or *KIT* genes.[40] In contrast to the aggressive clinical course of TNBCs, most salivary gland–type malignancies of the breast behave as low-grade neoplasms.

Molecular Classification

Breast cancers show great variation in gene expression patterns. In 2000, Perou et al[41] described four intrinsic subtypes of breast cancer using microarray gene analysis: luminal, ERBB2 positive, normal breast, and basal-like. An additional intrinsic subtype, claudin-low, was later identified in both mouse and human breast tumors.[42] Claudin-low tumors are typically triple-negative invasive ductal carcinomas and are characterized by decreased expression of genes coding for proteins involved in tight junctions and cell-to-cell adhesion. The proteins that show a decreased expression in claudin-low tumors include E-cadherin, occludin, and claudins 3, 4, and 7. This decrease is accompanied by increased messenger RNA (mRNA) expression of known transcriptional repressors of E-cadherin such as snail homolog 1 (*SNAI1*), snail homolog 2 (*SNAI2*), twist basic helix-loop-helix transcription factor 1 (*TWIST1*), twist basic helix-loop-helix transcription factor 2 (*TWIST2*), zinc finger E-box binding homeobox 1 (*ZEB1*), and zinc finger E-box binding homeobox 2 (*ZEB2*).[43] Despite the low expression of E-cadherin, these tumors are histologically not similar to invasive lobular carcinomas. Claudin-low tumors show epithelial to mesenchymal transition (EMT) features and a high expression of hypoxia-inducible factor 1a, immune system responses, and stem cell–associated biological processes.

Lehmann et al[44] reported gene expression profiles from 21 publicly available data sets containing 3,247 primary human breast cancers. Seven triple-negative subtypes were identified by consensus clustering, gene ontology, and differential gene expression studies: basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL), luminal androgen receptor (LAR), and unstable. Further independent analysis narrowed this to four clusters.[44] The gene expressions of these four clusters were similar to those of BL1 and BL2, IM, M and MSL, and LAR subtypes.

The BL1 and BL2 subtypes of TNBC are heavily enriched with genes involved in cell cycling, cell division, cell proliferation, the DNA damage response, growth factor signaling pathways, glycolysis, and gluconeogenesis.[44] Higher expression of *TP63* and membrane metalloendopeptidase (*MME*) genes indicate a basal/myoepithelial origin and the luminal progenitor stage of cell differentiation.[44]

The IM subtype of TNBC is enriched for genes involved in immune cell processes.[44] These processes include immune cell and cytokine signaling, such as the T helper 1, T helper 2, natural killer cell, B-cell receptor, and dendritic cell pathways. Genes involved in antigen processing, presentation, and signaling through core immune pathways, such as the nuclear factor of κ light polypeptide gene enhancer in B cells, tumor necrosis factor, and Janus kinase/signal transducer and activator of transcription pathways, are also enriched. The classification of this subtype has met with some skepticism. Critics believe that instead of being derived from tumor cells, the cells responsible for this gene expression are part of the tissue microenvironment and should therefore not be used for identifying a distinct subtype.[45]

The M and MSL subtypes of TNBC are heavily enriched in pathways involving cell motility, differentiation, and growth, including the wingless-type MMTV integration site family (WNT), anaplastic lymphoma kinase, and transforming growth factor beta (TGF-β) signaling pathways.[45] Unlike the M subtype, MSL also expresses additional genes linked to growth factor signaling pathways, including inositol phosphate metabolism, EGFR, platelet-derived growth factor, G-protein coupled receptor, and extracellular signal-regulated kinases 1 and 2 signaling. The MSL subtype expresses low levels of

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proliferation genes and claudins 3, 4, and 7; it likely correlates with the claudin-low intrinsic subtype of breast cancer. These claudin-low tumors have increased expression of genes associated with EMT and are believed to be derived from the mammary stem cell.[43]

The LAR subtype has the most diverse gene expression of the TNBC subtypes and corresponds to the late luminal stage of cell differentiation.[43,44] Despite being ER negative, hormonally regulated pathways, such as steroid synthesis, porphyrin metabolism, and androgen/estrogen metabolism, are heavily enriched in this subtype. The androgen receptor (AR) is highly expressed, with mRNA levels approximately nine times greater and intensity of immunohistochemical staining for AR more than 10 times greater than the other TNBC subtypes.

Table 1 provides a brief summary of the epidemiologic, radiologic, morphologic, immunohistochemical, and molecular features of TNBC, which can serve as a quick reference for the material covered this far.

Biomarkers and Their Prognostic and Therapeutic Potential

The Oncotype Dx (Genomic Health, Redwood City, CA) and Mammaprint (Agendia NV, Amsterdam, The Netherlands) molecular tests that are in current clinical use are generally used for ER-positive tumors to guide treatment decisions,[46] but these tests do not provide specific benefit for patients with TNBC.[47] An additional test that has been adopted for clinical use, PAM50 (NanoString Technologies, Seattle, WA), provides the intrinsic subtype classification and predicts relapse-free survival and likelihood of recurrence.[48] Further discovery and validation of biomarkers to serve as prognostic aids and as potential therapeutic targets are necessary to advance the treatment of patients with TNBC.

Androgen Receptor

AR is coexpressed with ER in the majority of breast cancers. AR inhibits proliferative activity in ER-positive tumors,[49] but it acts independently to promote tumorigenesis in an androgen-dependent manner in ER-negative tumors.[50] AR can be present independent of ER, with one recent review estimating that AR is expressed in approximately 30% of TNBCs.[51] AR mediates the ligand-dependent activation of the WNT and HER2 signaling pathways, which is accomplished through direct transcriptional induction of *WNT7B* and *HER3*. Specific targeting of the AR with bicalutamide (marketed under various names including Casodex [AstraZeneca, Wilmington, DE], Cosudex, Calutide, and Kalumid) inhibited the growth of dihydrotestosterone-stimulated ER-negative/HER2-positive tumors in vivo.[52] Bicalutamide efficacy was

Table 1
Characteristics of TNBC

Epidemiology	12%-24% of breast cancers Young patient population African American women and women of West African ancestry are at increased risk Aggressive behavior compared with non-TNBC Higher likelihood of distant metastasis Quicker relapse time
Imaging	18% of TNBC occult on mammography 92%-100% sensitivity with ultrasonography MRI is most sensitive (99%-100%) Combined use of mammography, ultrasonography, and MRI provides the greatest sensitivity but with increased false-positives and decreased positive predictive value
Morphology	High-grade ductal carcinoma, no special type is most common (80%-93%) Less frequent histologic patterns include metaplastic, medullary, and salivary gland–like carcinomas Common histologic features include Pushing border Cellular fibrous proliferation Lymphocytic infiltrate within the tumor and in lobules adjacent to the tumor
Immunohistochemistry	Always negative: ER, PR, HER2 May be positive: CK 5/6, EGFR
Intrinsic molecular classification	Basal-like (most common) Claudin-low Luminal subtypes make up a small minority
TNBC genomic subtypes	Basal-like 1 Basal-like 2 Immunomodulatory Mesenchymal Mesenchymal stem-like Luminal AR Unstable

AR, androgen receptor; CK, cytokeratin; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; MRI, magnetic resonance imaging; PR, progesterone receptor; TNBC, triple-negative breast cancer.

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tested on TNBC cell lines, of which the LAR subtype cell lines were significantly more sensitive than the other subtypes.[44] Although the laboratory results are encouraging, the effects of AR antagonists in patients with breast cancer are currently unknown. Phase 2 trials with the AR antagonists bicalutamide and enzalutamide (Xtandi, Astellas Pharma, Northbrook, IL) are currently ongoing. These studies include patients with AR-positive/ER-negative/PR-negative tumors and AR-positive/TNBCs, respectively.

Epidermal Growth Factor Receptor

EGFR is a type I transmembrane tyrosine kinase in the same family as HER2.[53] Dysregulation of EGFR and its pathway has been reported in numerous epithelial tumors, with a frequency of dysregulation greater than 50% in TNBC and 65% to 72% in basal-like carcinomas reported in some studies.[54] However, other studies have produced discordant findings. One study of TNBCs found that none of the 84 tumors evaluated exhibited *EGFR* gene amplification.[55] The *EGFR* gene is not enriched in all basal-like tumors but in the BL2 subtype alone.[44] It is also enriched in a minority of the mesenchymal subtypes. Lapatinib (Tykerb/Tyverb, GlaxoSmithKline, Middlesex, England), a dual EGFR/HER2 kinase inhibitor, has been tested as monotherapy and in combination with other therapies for TNBCs. Unfortunately, these treatments have met with limited success. In one study, the addition of lapatinib to a paclitaxel regimen (Taxol, Bristol-Myers Squibb, New York, NY) had a negative impact on clinical outcomes with a shorter median event-free survival in triple-negative and HER2/PR-negative patients.[56] Another study demonstrated increased aggressiveness of TNBCs in mouse models treated with lapatinib.[57] Targeting EGFR may also be rendered difficult because the expression of EGFR in primary breast cancers vs metastatic deposits is discordant.[58] Although lapatinib is currently approved by the Food and Drug Administration (FDA) only for the treatment of certain advanced HER2-positive breast cancers, the emerging information about TNBC subtypes may be the grounds for evaluating its efficacy in select TNBC cases.

Fibroblast Growth Factor/Receptor

TNBCs often demonstrate a cellular fibrous proliferation (Image 2C and Image 2D), and the role of the fibroblast growth factor/receptor (FGF/R) pathway in TNBCs is an active area of research. The FGF/R pathway is fundamental to a wide variety of physiologic processes including cell proliferation, survival, and migration. Proliferation is accomplished primarily through the RAS-RAF-mitogen-activated protein kinase (MAPK)/ERK cascade. Antiapoptotic pathways are activated through the phosphoinositide 3-kinase (PI3K)/v-akt murine thymoma viral oncogene homolog (AKT)/mammalian target of rapamycin (mTOR) cascade.[59] In a study by Turner et al,[60] a subgroup of TNBC cell lines with *FGFR2* mutations displayed constitutive activation of *FGFR* and were highly susceptible to FGFR inhibitors. Amplified cell lines were also highly susceptible to FGFR inhibition, which induced apoptosis. The *FGFR2* gene was amplified in 4% of TNBCs but not in receptor-positive breast cancers.[60] The mesenchymal-like types of TNBCs have shown increased expression of the FGF pathway.[44]

FGFR1 has been identified in triple-negative cell lines. Amplification of *FGFR1* recruits macrophages to the tumor microenvironment. There is a strong correlation between macrophage density and poor prognosis. Recruitment of macrophages leads to the promotion of cell invasion, angiogenesis, and immune suppression.[61] The invasion and migration of tumor cells is made possible by the decreased activity of the TGF-β/SMAD3 pathway, which contributes to the increased activity of chemokine (C-X-C motif) receptor 2 (CXCR2)–binding chemokine. It is through this activation of CXCR2 that macrophages are capable of promoting tumor extravasation and migration. Targeting CXCR2 may present a possible therapeutic target.[62] Studies examining this are currently in the preclinical stages. FGFR-induced inflammatory chemokine (C-X3-C motif) ligand 1 (CX3CL1) recruits chemokine (C-X3-C motif) receptor 1 (CX3CR1)–expressing macrophages, resulting in increased angiogenesis. The tumor microenvironment has been well established as an influence on tumor progression,[63] and it appears that the FGF/R pathway is integral to this relationship. Ongoing clinical trials are studying the effects of FGFR inhibitors in patients with breast cancer. One such trial showed that targeting FGFR could lead to modest antitumor activity in patients with FGF-pathway–deregulated breast cancer.[64]

Vascular Endothelial Growth Factor/Receptor

TNBCs are often highly vascular (Image 2E and Image 2F). Neoangiogenesis is crucial to tumor progression and is the result of several mechanistic processes including the vascular endothelial growth factor (VEGF) pathway.[65] Compared with non–triple-negative cancers, TNBCs have significantly higher levels of VEGF, and these levels correlate with poor outcome regardless of tumor stage.[66] Many factors are involved in the production of VEGF.[67] Hypoxia-induced production of VEGF is mediated by the binding of hypoxia-inducible factor 1 (HIF1). Numerous growth factors and cytokines also stimulate VEGF production including epidermal growth factor, transforming growth factor, insulin-like growth factor, interleukin 1α, and interleukin 6. During breast carcinogenesis, HIF1 increases proportionally in the progression from ductal hyperplastic lesions to high-grade invasive ductal carcinoma.[68]

In 2008, the FDA approved bevacizumab (Avastin, Genentech, South San Francisco, CA), a VEGF inhibitor, in combination with paclitaxel as a first-line treatment for

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metastatic HER2-negative breast cancer based on the Eastern Cooperative Oncology Group 2100 trial.[69] This approval was revoked in 2011 after subsequent trials (AVADO and RIBBON-1 trials) failed to show a significant benefit in overall survival despite improved clinical surrogate endpoints, such as progression-free survival.[70] The hazard ratios for time to progression of disease among patients with TNBC were similar to those for patients with hormone-receptor–positive tumors in all three of these trials, but the absolute gains were smaller because of the more rapid growth rates of TNBC. This decision remains controversial because bevacizumab is still approved in several other countries as a first-line treatment. A meta-analysis of phase III trials with bevacizumab as first-line treatment for metastatic breast cancer demonstrated the drug's efficacy with significantly improved overall tumor response rate and progression-free survival. However, no significant improvement in overall survival was observed, and there was a significant increase in grade 3 to grade 4 toxicities.[71]

The VEGF receptor is another potential therapeutic target. The three membrane-bound VEGF receptors are tyrosine kinase receptors. VEGF receptor 2 is the primary mediator of angiogenesis. Sunitinib (Sutent, Pfizer, New York, NY) is a tyrosine kinase inhibitor that targets VEGF receptors 1, 2, and 3, platelet-derived growth factor receptor, KIT, FMS-like tyrosine kinase 3 (FLT3), and ret proto-oncogene (RET). Despite early promise, phase III trials have been largely disappointing.[72,73] Sorafenib (Nexavar, co-developed by Bayer [Pittsburgh, PA] and Onyx Pharmaceuticals [San Francisco, CA]) is another tyrosine kinase inhibitor having multiple specificities and is FDA approved for renal and hepatic malignancies. In a series of four phase IIb trials, sorafenib was administered in combination with select chemotherapies to patients with advanced HER2-negative breast cancer. Improvements were noted in progression-free survival when sorafenib was administered with capecitabine (Xeloda, Genentech) and/or gemcitabine (Gemzar, Eli Lilly & Co, Indianapolis, IN).[74] In one of these trials (SOLTI-0701), a prespecified subgroup analysis of patients with TNBC showed an improvement in median progression-free survival of almost 2 months with the addition of sorafenib to capecitabine.[75] The AC01B07 trial showed a trend toward improvement in progression-free survival in patients with TNBC treated with sorafenib in combination with gemcitabine or capecitabine.[75] Other VEGF receptor inhibitors are in phase I or II trials.

One difficulty encountered in trials of FGFR, EGFR, and VEGF receptor inhibitors is the lack of biomarkers that indicate whether a tumor is susceptible to a particular tyrosine kinase inhibitor. A tumor that is known to express a particular tyrosine kinase receptor may not necessarily respond to an inhibitor that is targeted for that receptor. In addition, constitutive activation of downstream pathways may render tyrosine kinase inhibitors irrelevant.

PI3K/AKT/mTOR

TNBCs have demonstrated a high incidence of clonal mutations in *P53, PI3K,* and *PTEN*[76] and heavy gene enrichment of at least one of these genes across all TNBC subtypes.[44] Montero et al[77] observed frequent coactivation of various tyrosine kinase receptors and frequent activation of both the PI3K/AKT/mTOR and the MAPK/ERK pathways in a set of TNBCs. Pharmacologic inhibition studies showed that agents that target the mTOR pathway have more potent and efficient antitumoral effects than agents that target tyrosine kinase receptors.

mTOR is a serine-threonine protein kinase that exists in two forms called mTOR complexes 1 and 2 (mTORC1 and MTORC2). It is regulated through the PI3K/AKT pathway. mTORC1 consists of the mTOR protein, mammalian LST8 (mLST8), proline rich AKT substrate 40 (PRAS40), and raptor. The downstream effects of the activation of this complex have been associated with cellular transformation, and their overexpression has been linked to a poor prognosis in cancer.[78]

mTORC2 consists of mTOR, mLST8, rapamycin-insensitive companion of mTOR (rictor), MAPK-associated protein 1 (mSIN1), and protein observed with rictor (protor).[79] mTORC2 phosphorylates AKT on serine 473 and also regulates integrin-linked kinase promotion of AKT phosphorylation.[80] Although AKT generally has an inhibitory effect on multiple targets, most of these targets are negative regulators. Thus, the net result is cellular activation. AKT phosphorylates downstream mediators controlling transcription, cell cycle progression, metabolism, and cell survival. By regulating phosphorylation of protein kinase C alpha (PKCα) and control of the actin cytoskeleton, mTORC2 plays a role in cell migration.

There is some evidence that mTOR inhibitors may have a role in TNBCs.[81,82] A phase II study showed that the addition of an mTOR inhibitor to standard chemotherapy in TNBC resulted in a small improvement in the 12-week response rate and was well tolerated by patients.[83] Additional trials are ongoing.

Treatment Options

The pursuit of novel molecular therapies for TNBC has been vigorous, but as of now, surgery remains the best modality for local control of TNBC. A meta-analysis revealed a significantly higher rate of local recurrence among triple-negative tumors compared with the luminal subtypes. The type of surgery, breast-conserving or total mastectomy, had no significant impact on the rate of locoregional recurrence.[84]

TNBCs are insensitive to some of the most effective therapies for non-TNBCs, which include endocrine and HER2-directed therapies. Cytotoxic chemotherapy is currently the

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mainstay for TNBC. Several studies have demonstrated that TNBCs have a higher pathologic complete response (pCR) rate than hormone receptor–positive breast cancers when treated with neoadjuvant chemotherapy. The efficacy of neoadjuvant therapy was conclusively demonstrated in a prospective study of 1,118 patients between 1985 and 2004 at the MD Anderson Cancer Center (Houston, TX)[85]; a pCR was seen in 22% of patients with TNBC compared with 11% of patients with non-TNBC. However, patients with TNBC had significantly worse 3-year progression-free and 3-year overall survival rates, highlighting the relatively poor prognosis of this disease. This is the "triple-negative paradox." Within all types of TNBCs, those with a high proliferative index, as measured by nuclear positivity with immunostaining for antigen identified by monoclonal antibody Ki-67 (Image 1I), demonstrate a higher pCR but have a lower recurrence-free survival. TNBCs with a lower proliferation rate tend to have a better prognosis.[86]

Current systemic chemotherapy consists largely of third-generation adjuvant or neoadjuvant regimens. These regimens are anthracycline/cyclophosphamide based, combined with a taxane.[87] Numerous studies have shown an increased pCR and disease-free survival rate with the addition of taxanes to anthracycline-based chemotherapy.[88,89]

Because of the improved management of side effects and new preclinical data, platinum agents have seen a renewed interest. Platinum agents act through the formation of DNA crosslinks, resulting in double-stranded DNA breaks. In a *BRCA*-mutated tumor, the absence of homologous recombination prohibits error-free repair, and this leads to cell death. High-dose platinum therapy has shown a greater benefit than conventional chemotherapy for *BRCA1*-like tumors, with one study demonstrating an eightfold decrease in the risk of recurrence. In the TNBC group, this difference was replicated between the *BRCA*-mutant and non-mutant TNBCs.[90] In another study, lower *BRCA1* mRNA expression was associated with a significantly greater response to platinum-based treatment.[91] Interestingly, platinum agents have also been shown to benefit wild-type *BRCA1* cancers by inactivating the E3 ubiquitin ligase activity of the BRCA1-BRCA1–associated RING domain 1 (BARD1) complex.[92]

Poly(ADP-ribose) polymerase inhibitors (PARP inhibitors) have been a focus of interest for treating TNBC. PARPs produce large chains of poly(ADP)ribose from nicotinamide adenine dinucleotide. PARP-1 plays a key role in the response to DNA damage, especially single-strand breaks, through the base excision repair pathway. In the presence of a PARP inhibitor in *BRCA*-mutated cells, stalled replication forks result, and accumulated DNA damage either remains unrepaired or is repaired by error-prone mechanisms.[93] Trials with PARP inhibitors showed early success in phase 1 and 2 clinical trials,[94-96] but this success was not replicated in a subsequent phase 3 trial.[97] There are several possible explanations for this inconsistency. Patient selection is one plausible explanation. Instead of including only *BRCA*-mutant tumors, the phase 3 trial included all tumors that were triple negative on immunohistochemistry. Although the majority of TNBCs are basal-like, the claudin-low group, comprising 25% to 39% of TNBCs, is less likely to harbor *BRCA* mutations.[43] The impact of PARP inhibitors may depend on the class of chemotherapeutic agents with which it is being combined, and this may be related to alterations in the mechanism of DNA repair.[98]

TNBC Recurrence and Metastasis

Although TNBCs tend to have a good initial response to treatment, they recur more quickly than other types of breast cancer, generally within 1 to 3 years. Distant recurrence is more frequent than local recurrence, and median survival from the time of recurrence is 9 months.[8] Tumor heterogeneity may pose a confounding challenge (Image 3B, Image 3C, and Image 3D). In 2010, Ding et al[99] screened the entire genome in four samples from a patient with a basal-like breast cancer. The four samples examined included the primary basal-like breast cancer, peripheral blood, a cerebellar metastasis, and a xenograft from the primary tumor. This revealed 28 deletions and six inversions as somatic events. There was considerable genetic heterogeneity in the cells of the primary tumor. Among the deletions was a heterozygous deletion in F-box and WD repeat domain containing 7,E3 ubiquitin protein ligase (*FBXW7*), which targets cyclin E and mTOR for ubiquitin-mediated degradation. The loss of *FBXW7* causes chromosomal instability and tumorigenesis. Other significant mutations included catenin (cadherin-associated protein) α1 (*CTNNA1*), resulting in global loss of cell adhesion in human breast cancer cells, and neuregulin 1 (*NRG1*), which encodes a growth factor that binds to ERBB3 and ERBB4. The metastatic tumor had additional mutations and a large deletion.[99]

A prospective study of metastatic TNBC specimens uncovered somatic mutations using whole genome and transcriptome sequencing.[100] The tumors revealed remarkable heterogeneity. Of the 14 tumors studied, eight had expression profiles indicative of the basal-like subtype of breast cancer. The expression profile in these eight cases was heavily enriched in genes involved in cell cycle control, G_2-M checkpoint regulation, and mitosis. One of these, the *forkhead box M1* (*FOXM1*) gene, encodes a transcriptional activator that regulates many of the genes involved in cell proliferation processes, such as aurora kinase A and B (*AURKA*, *AURKB)*, polo-like kinase 1 (*PLK1*), and centromere protein F (*CENPF*). Tumor suppressor genes, such as *TP53*, retinoblastoma 1 (*RB1*), and *PTEN*, were also well

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represented. The analysis also revealed unique amplified double minutes containing a v-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutation. Of note, nine of the 14 patients had alterations that converged on the RAS/RAF/mitogen-activated protein kinase kinase (MEK)/ERK and PI3K/AKT/mTOR pathways. The sequencing results were used to guide the treatment of the study patients with targeted therapy consisting of agents such as PI3K/mTOR inhibitors, PARPs, anti-VEGFs, and MEK inhibitors. Two of the 14 patients achieved a complete response, five achieved a partial response, and three had stable disease following treatment. Considering the dismal prognosis of metastatic TNBC thus far, these results are encouraging.

Conclusion

TNBC has the reputation of being an aggressive form of breast cancer that affects young women and lacks targeted therapy. However, as our knowledge of these tumors has expanded, it has become clear that this is a heterogeneous group that encompasses a wide range of clinical outcomes. TNBC has histologic subtypes that range from salivary gland–type tumors with low-grade histologic features and low-grade behavior, to medullary carcinomas with high-grade histologic features but less aggressive behavior, to tumors with high-grade histologic features and aggressive clinical behavior.

Through the application of genomic and molecular techniques, the molecular heterogeneity of breast cancer, particularly TNBC, has become evident with subtypes such as BL, IM, M, and LAR type. Each demonstrates a unique pattern of gene expression. As the genetic and molecular profiles of TNBC are elucidated, a number of therapeutic targets have been identified.

TNBCs are negative for ER, PR, and HER2; therefore, they have not benefitted from therapies directed toward these well-known biomarkers. However, now a multitude of biomarkers are on the horizon, which can be exploited to yield similar prognostic information and survival benefits for TNBC. Thus far, agents directed at VEGF and PARP have produced mixed results. However, researchers have identified other biomarkers and pathways involved in TNBC tumorigenesis (eg, AR, EGFR, FGFR, CXCR2, VEGFR, and the mTOR pathway) that may be amenable to therapeutic intervention; clinical trials targeting them are under way. For patients and families as well as physicians treating this cancer, the therapeutic benefits of these cannot come soon enough.

Address reprint requests to Dr Singh: Stony Brook University School of Medicine, Health Sciences Tower, Level 4, Room 184, Stony Brook, NY 11794-8430; meenakshi.singh@stonybrookmedicine.edu.

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