Targeting the Molecular Subtypes of Triple Negative Breast Cancer: Understanding the Diversity to Progress the Field

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Key Words. Triple negative breast cancer • Molecular subtypes • Gene expression profiling • Targeted therapy

ABSTRACT

Triple negative breast cancers (TNBCs) represent 10%–20% of primary breast cancers, and despite having greater initial sensitivity to cytotoxic chemotherapy, patients with TNBCs have higher rates of distant metastasis and a poorer prognosis compared with patients with hormone receptor positive and/or human epidermal growth factor receptor 2 positive disease. TNBC has historically been treated as a single disease entity in targeted therapy trials, but advances in gene expression profiling and other molecular diagnostic techniques over the last decade have revealed considerable biologic heterogeneity within TNBCs, including subgroups with distinct, targetable aberrations. Such molecular heterogeneity explains, in part, the disappointing performance of targeted therapeutics in unselected TNBC. Here we discuss the history of gene expression profiling in breast cancer and its application in partitioning TNBCs into subtypes that may lead to more consistent therapeutic successes in this heterogeneous disease.

Implications For Practice: Triple negative breast cancers (TNBCs) have historically been regarded as a single entity in clinical trial design. Over the last decade, molecular characterization has revealed much heterogeneity in TNBCs, explaining in part the lackluster performance of targeted therapeutics in TNBCs as a group. In this article, we review the history of the molecular classification of breast cancer based on gene expression profiling and discuss its role in TNBCs.

INTRODUCTION

Triple negative breast cancers (TNBCs) account for approximately 10%–20% of primary breast cancers [1–4] and are characterized by a lack of expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Compared with their ER/PR positive and HER2 positive counterparts, TNBCs are, in general, larger, of higher grade, and more likely to be node positive [5]. Although more likely to respond to neoadjuvant chemotherapy, chemotherapy-insensitive TNBC is associated with a poorer prognosis compared with other breast cancer subtypes for which targeted therapy either enhances chemotherapy effect or treats chemo-insensitive disease [5–7].

Despite a plethora of valid basic science research supporting the use of targeted therapy in TNBC and numerous clinical trials of active targeted agents for the treatment of TNBC, not a single targeted therapy has been U.S. Food and Drug Administration (FDA)-approved for the treatment of this particularly aggressive form of breast cancer. In addition to the known challenges for developing targeted agents in cancer such as clonal selection of resistant cells or activation of “escape” pathways, drug development in TNBC has further complexities that are inherent to clinical trial design rather than disease resistance. The lack of consistent success in the treatment of TNBC has been attributed in part to the underlying molecular heterogeneity of TNBCs.

Recent advances in gene expression profiling have identified subgroups of TNBC with distinct molecular features that, if appropriately selected, may be more responsive to targeted therapy with existing FDA-approved drugs, leading to rapid improvement of outcomes in this high-risk breast cancer population [8–11]. Here we review recent attempts to classify TNBCs into various subtypes and their implications for the development of targeted therapies.

HISTORY OF GENE EXPRESSION PROFILING IN TNBC

Historically, breast cancers have been divided into subtypes based on differential expression of ER/PR, and subsequently HER2 by immunohistochemistry (IHC). The emergence of more sophisticated techniques in molecular biology in the late 1990s led to the use of in situ hybridization as an adjunctive test of
HER2 status by quantifying gene amplification. More recently, gene expression profiling has been used to identify subtypes in breast cancer with a focus on TNBC where no clinically actionable subtypes currently exist (Fig. 1).

One of the earliest signs of heterogeneity of gene expression profiles in breast cancer was described by Perou et al. [12] when they, through the use of cDNA microarrays, subclassified breast cancer into four broad clusters based on their gene expression profiles: (a) luminal/ER gene cluster, (b) ERBB2 overexpression cluster (ERBB2+), (c) basal epithelial associated cluster, and (d) normal-breast-like cluster.

The luminal subtype displayed patterns of gene expression reminiscent of luminal epithelial cells, including cytokeratins 8/18, ER, and other genes associated with ER activation [13]. A subsequent analysis then revealed that the luminal subtype could be further divided into at least two different subtypes based on differential expression of luminal specific genes, including the ER cluster of genes, giving rise to the terms “luminal A” and “luminal B” [14]. Consequently, these five distinct molecular subtypes of breast cancer (luminal A, luminal B, ERBB2+, basal-like, normal breast-like) became what is now commonly known as the “intrinsic” molecular subtypes of breast cancer (Fig. 1).

The ERBB2 overexpression cluster had a characteristic overexpression of genes located in the same region of chromosome 17 as the ERBB2 locus [12]. Although clinically HER2-positive tumors (by IHC or fluorescent in situ hybridization) are sometimes referred to interchangeably with tumors from the ERBB2 overexpression cluster, this relationship is not exact because clinically HER2-positive tumors that are also hormone receptor positive may have gene expression profiles that more closely resemble those in the luminal subtypes [14–16].

In contrast to the luminal and ERBB2+ subtypes, the basal epithelial associated cluster comprised tumors that had expression profiles similar to basal epithelial cells [12], characterized by the lack of expression of ER and HER2, overexpression of basal cytokeratins, and proliferation-related genes [12, 15]. Although breast cancers with a basal-like gene expression profile tend to be TNBCs [17, 18], the converse is not true, as there is still considerable heterogeneity in gene expression profiles within TNBCs [19, 20].

Finally, the normal-breast-like cluster was characterized by high expression of basal epithelial and adipocyte-associated genes and low expression of luminal epithelial-associated genes. In the study by Perou et al., a single fibroadenoma, three normal breast specimens, and several tumor samples were assigned to this cluster [12].

Later, Prat et al. [21] described the phenotypic and molecular characteristics of yet another subtype—the claudin-low tumors, which are characterized by low expression of tight junction proteins (claudin 3, 4, and 7, as well as E-cadherin). 61%–71% of claudin-low tumors are TNBCs with a relatively high frequency of metaplastic or medullary differentiation. These tumors also demonstrated a cell surface marker expression pattern similar to mammary stem cells and breast tumor-initiating cells.

The identification of these “intrinsic” subtypes of breast cancer led to several studies evaluating the impact of specific subtypes on prognosis [14, 16, 22] and response to chemotherapy [23], in which the luminal subtypes were found to have a more indolent course and the basal subtype, while associated with poorer prognosis, had higher response rates to chemotherapy. Parker et al. developed a standardized method of identifying the “intrinsic” subtypes of breast cancer by applying a Prediction Analysis of Microarray (PAM) algorithm to a 50 gene set, commonly known as PAM50 [24], which has been used in a variety of investigational settings. A recent multicenter phase II trial of platinum monotherapy in metastatic TNBC showed a trend toward increased objective response rate (ORR) in basal versus nonbasal TNBC as defined by PAM50, but this was not statistically significant [25]. In the neoadjuvant setting, retrospective molecular analysis of pretreatment tumor samples obtained during the CALGB 40603 study showed that the benefit of adding carboplatin was consistent across all PAM50 subtypes, including nonbasal TNBCs [26, 27]. Therefore, at this point in time, the commercially available PAM50 assay has limited utility in guiding therapy for most cases of TNBC.

In an effort to better understand the heterogeneity of TNBCs, Lehmann et al. [9] analyzed gene expression profiles of...
Table 1. Distribution of molecular subtypes in triple negative breast cancer

<table>
<thead>
<tr>
<th>TNBC molecular subtypeb</th>
<th>Intrinsic molecular subtypea</th>
<th>Basal-like</th>
<th>ERBB2+</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>Normal breast-like</th>
<th>Unclassified</th>
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<td>9</td>
<td>14</td>
<td></td>
<td>18</td>
<td>78</td>
</tr>
<tr>
<td>IM</td>
<td>61</td>
<td>9</td>
<td>11</td>
<td>6</td>
<td>5</td>
<td>24</td>
<td></td>
<td>116</td>
</tr>
<tr>
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<td>7</td>
<td>43</td>
<td>6</td>
<td>2</td>
<td></td>
<td>4</td>
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<td>26</td>
<td></td>
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<td>0</td>
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<td>3</td>
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<td>90</td>
<td>42</td>
<td>75</td>
<td></td>
<td>90</td>
<td>586c</td>
</tr>
</tbody>
</table>

*aIntrinsic molecular subtype as defined by [14].

*bTNBC molecular subtype as defined by [9].

*cOf the 587 TNBC samples evaluated by [9], information on the corresponding intrinsic subtype for 586 samples were available in the supplementary material.

Abbreviations: BL1, basal-like 1; BL2, basal-like 2; ERBB2+, ERBB2 overexpression cluster; IM, immunomodulatory; LAR, luminal androgen receptor; M, mesenchymal; MSL, mesenchymal stem-like; TNBC, triple negative breast cancer.

587 TNBCs across 21 breast cancer data sets through cluster analysis based on differential expression of a set of 2,188 genes and identified six stable TNBC subtypes, including two basal-like (BL1 and BL2), an immunomodulatory (IM), a mesenchymal (M), a mesenchymal stem-like (MSL), and a luminal androgen receptor (LAR) subtype. Later, this group used histopathological quantification and laser capture microdissection to demonstrate that transcripts used to define the IM and MSL subtypes were from tumor-infiltrating lymphocytes and peritumoral stromal cells, respectively [28]. This group also characterized TNBC cell lines based upon these signatures and demonstrated that BL1 and BL2 subtypes preferentially responded to cisplatin, whereas the M and MSL subtypes responded to inhibition of the phosphoinositide 3-kinase (PI3K)/mTOR and Abl/Src pathways, and the LAR subtype was exquisitely sensitive to AR antagonism, suggesting that gene expression analysis could help match patients with TNBCs to appropriate targeted therapies.

Subsequently, Burstein et al. analyzed RNA and DNA profiling from 198 TNBC tumors and, like Lehmann et al., identified distinct LAR, mesenchymal, and basal subtypes; however, they proposed the segregation of the basal-like subtype into basal-like immune suppressed (BLIS) and basal-like immune activated (BLIA) subtypes [29]. This alternate classification was proposed in part because the BL1 and BL2 subtypes proposed by Lehmann et al. were not readily distinguishable using hierarchical clustering of public TNBC data sets. The proposed BLIS subtype exhibited downregulation of B cell, T cell, and natural killer cell immune-regulating pathways and cytokine pathways and had the worst prognosis. In contrast, the BLIA subtype had an upregulation of immune-associated pathways and had the best prognosis.

**TARGETING THE SUBTYPES OF TNBC FOR THERAPEUTIC BENEFIT**

**Basal-Like Triple Negative Breast Cancers**

Early studies of gene expression profiling in breast cancer demonstrated that approximately 60%–72% of TNBCs and 80%–90% of breast cancers in patients with germline *BRCA1* mutations had a basal-like pattern of gene expression [19, 25, 30, 31]. This phenotypic similarity led to the hypothesis that defects in homologous recombination-mediated DNA repair pathways were central to the development of basal-like TNBCs, suggesting that agents that exploited this deficiency could potentially be successful in the clinic [32]. A single arm phase II study evaluating neoadjuvant single-agent cisplatin in patients with TNBC reported a pathologic complete response (pCR) rate of only 22% despite the fact that all patients on this study had a basal-like gene expression profile [33]. In addition, because it was found that the epidermal growth factor receptor (EGFR) was overexpressed in basal-like breast cancer [16, 18, 34], anti-EGFR therapy was thought to hold promise for TNBC and, in particular, the basal-like subtype. In a randomized phase II study of cetuximab in combination with carboplatin in metastatic TNBC, response rates to the combination was reported to be 17%. However, in the subset of patients who had basal-like tumors, the response rate to the combination was 8% [35]. Together, these observations suggest a greater degree of molecular heterogeneity in the basal-like subtype of TNBCs than was initially appreciated. Notably, in the study by Lehmann et al. [9], TNBC samples that would have been classified as basal-like based on the intrinsic molecular classification system proposed by Sorlie et al. [14, 22] were found not only in the BL1 and BL2 subtypes, but also in the IM, M, MSL and unstable subtypes, providing further evidence of heterogeneity within the original basal-like subtype (Table 1). BL1 TNBCs make up 18% of TNBCs and are characterized by high levels of expression of genes involved in the cell cycle and DNA-damage repair pathways. In contrast, BL2 TNBCs, which represent 13% of TNBCs, demonstrate upregulation of growth factor signaling pathways, including the epidermal growth factor (EGF), nerve growth factor (NGF), and MET pathways, as well as genes involved in glycolysis and gluconeogenesis. It therefore follows that BL1 TNBCs should demonstrate greater sensitivity to strategies targeting the DNA-repair pathways such as platinum-based chemotherapy and poly-ADP ribose polymerase (PARP) inhibition, whereas BL2 TNBCs should, theoretically, respond better to small molecule inhibitors of growth factor pathways. A study by Ueno et al. [36] showed that BL1 tumors had a pCR rate of 52% to neoadjuvant chemotherapy with anthracyclines.
and/or taxanes, whereas the pCR rate in BL2 tumors was 0%, providing orthogonal evidence that BL1 and BL2 are molecularly distinct entities that can be expected to respond differently to similar therapies.

**Mesenchymal and Mesenchymal Stem Cell-Like Triple Negative Breast Cancers**

The M and MSL TNBCs as defined by Lehmann et al. displayed upregulation of pathways involved in epithelial-to-mesenchymal transition (EMT). The M subtype is heavily enriched in pathways central to cell motility, extracellular matrix receptor interaction, and cellular differentiation. Although the MSL subtype is similarly enriched for pathways that are upregulated in the M subtype, expression of stem cell-associated genes, as well as genes involved in certain growth factor signaling pathways and angiogenesis, is uniquely associated with the MSL subtype. In addition, the MSL subtype also showed limited expression of claudins 3, 4, and 7, similar to the claudin-low subtype described by Prat et al. Mesenchymal TNBCs are enriched in epithelial-to-mesenchymal transition (EMT) and cancer stem-cell (CSC) features and contain a high rate of aberrations in the PI3K/AKT/mTOR pathway, raising the possibility of targeting this axis for the treatment of this subset [8, 9, 21, 37].

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As with all subtypes of TNBC, there is no clinical laboratory improvement amendments (CLIA)-certified diagnostic assay to identify mesenchymal TNBC, making patient selection for therapeutic trials challenging. Metaplastic breast cancer (MpBC) is a rare subtype of TNBC that can be clinically identified by light microscopy due to an admixture of epithelial and mesenchymal components within the tumor [38–44]. Approximately 10%–30% of TNBC tumors classified as mesenchymal by gene signature were found to be MpBCs based upon their morphologic features [21, 45]. Like mesenchymal TNBCs, MpBCs are often considered refractory to chemotherapy and have gene signatures that show enrichment in CSC and EMT features [8, 46–49]. MpBCs also have a high rate of aberrations in the PI3K/AKT/mTOR pathway and display high levels of angiogenesis characterized by expression of vascular endothelial growth factor and hypoxia-induced factor 1 alpha [50]. Given these features, MpBC may represent a “surrogate of response” for targeted therapy trials in mesenchymal TNBC [8]. Interestingly, a phase I study with dose expansion of liposomal doxorubicin, bevacizumab, and mTOR inhibition with temsirolimus or everolimus in patients with MpBC (n = 52) demonstrated an ORR of 21% with durable complete responses (CRs; 8%) and a clinical benefit rate (CBR; sum of CR, partial response [PR], and disease stability for greater than 6 months) of 40%. Of note, 74% of patients enrolled in this study had evidence of activating aberrations in the PI3K/AKT/mTOR pathway and the ORR was significantly higher in patients whose tumors had evidence of such aberrations [51].

Activation of the MET pathway has been associated with EMT and tumor progression [52], and although early results with c-MET-directed therapy have been disappointing in unselected TNBC patients with metastatic disease [53], tyrosine-protein kinase MET (c-MET)-targeted therapy could prove to be beneficial in patients who have the M or MSL subtype of TNBC. In addition, preclinical data have shown that inhibiting the transforming growth factor beta (TGF-β) receptor kinase can reverse EMT in vitro [54] and may represent a potential therapeutic opportunity. The NOTCH pathway has been implicated in the survival of stem cell-like initiating cells, and the MSL subtype may prove to be sensitive to NOTCH inhibition. A recent phase I study of the gamma secretase inhibitor, PF-03084014, in combination with docetaxel in unselected patients with advanced TNBC, reported that 16% and 44% of patients had a confirmed PR and stable disease (SD), respectively [55].

**Immunomodulatory Subtype**

While the hallmark of the IM subtype as described by Lehmann et al. is a shift towards the expression of genes involved in immune signaling pathways, it is unclear if this represents the true gene expression profile of tumor cells or if this is a mere reflection of a tumor which has a significant immune infiltrate [10]. Interestingly, the BLIA subtype described by Burstein et al. overexpressed cytotoxic T lymphocyte-associated protein 4 (CTLA-4) in addition to other immune related genes and was associated with better prognosis [29]. It is conceivable that there would be considerable overlap between tumors belonging to the IM and BLIA subtypes given the similarities in their gene expression profiles. We therefore hypothesize that the IM and BLIA subtypes would prove to be responsive to immune-based therapies such as check point inhibitors and tumor vaccines. Recently published results from the phase Ib study of pembrolizumab in patients with advanced TNBC reported an overall response rate of 18.5% [58]. This study selected patients on the basis of programmed death-ligand 1 positivity and no
data on gene expression profiling have been reported. As pembrolizumab moves forward in clinical development for TNBC, it will be interesting to determine if patients with the IM and/or BLIA subtypes will have better responses compared with patients with other subtypes of TNBC.

**HER2 Enriched Triple Negative Tumors**

Although TNBCs are by definition HER2 negative, a subset of TNBCs demonstrate a gene expression profile similar to the **ERBB2** overexpression cluster as described by Perou et al. [12]. This is an important consideration as centralized testing of tumor samples from the NSABP-B31 trial [59] showed that 10% of the patients treated with trastuzumab had tumors that lacked HER2 overexpression by IHC upon central review. Interestingly, trastuzumab appeared to have clinical benefit despite the absence of HER2 overexpression in a subset analysis of this patient population. Although not statistically significant, another subset analysis from a randomized phase II trial of the HER2 peptide vaccine AE37 showed a trend toward improved disease-free survival in TNBC patients with low-level HER2 expression who received the vaccine compared with those who did not [60]. Further data are needed to fully characterize the impact of HER2-directed therapies in TNBCs, and the ongoing phase III NASBP B47 trial should provide more clarity in this area. It is also plausible that gene signatures may one day aid in identifying TNBC patients who will benefit from HER2-directed therapies.

**Identification of Subtypes Based on Somatic and Germline Mutations**

With the widespread availability of high throughput sequencing, attempts to characterize TNBCs based on their somatic mutational landscape and potentially identify common targetable driver events have yielded some interesting observations. Not surprisingly, whole exome sequencing of genomic DNA from unselected TNBC cases at diagnosis demonstrated a wide variation in genomic evolution with some cases demonstrating low-clonality (fewer somatic mutations at higher allelic frequencies) and others showing evidence of more extensive clonal evolution (multiple somatic mutations at lower allelic frequencies) [61]. Although the relationship is not exact, basal-like TNBCs tend to have more extensive clonal evolution compared with non-basal-like TNBCs. **TP53** is the most commonly mutated gene in TNBC (60%–75%), but its role in predicting sensitivity to chemotherapy and long term outcomes in TNBC is controversial [25, 62]. The **PIK3CA** gene is another commonly mutated gene in TNBC (9%) [61], and efforts are underway to further define the role of PI3K inhibition in TNBC. While somatic mutations in **TP53, PIK3CA** and **PTEN** have been identified as clonally dominant genetic alterations in a substantial proportion of tumors, their clonal frequencies in a subset of tumors were inconsistent with founder status, suggesting variation in founder events resulting in carcinogenesis [61]. Despite current limitations, information derived from somatic mutation analysis of tumor specimens could complement the use of gene expression profiling in clinical practice. For example, Lehmann et al. reported that TNBC cell lines resembling the LAR and M/MSL subtypes demonstrated significant sensitivity to PI3K inhibition, which correlated with the presence of mutations in **PIK3CA** [9]. Currently ongoing trials are exploring the combination of AR blockade with PI3K inhibition, which may prove to be synergistic in patients with the LAR subtype of TNBC.

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In recent years, emerging data from clinical trials have suggested that the presence of germline mutations in **BRCA1** and **BRCA2** could potentially help inform treatment decisions. In the TNT trial, patients with metastatic or recurrent locally advanced breast cancer that was either triple negative or associated with germline mutations in **BRCA1** or **BRCA2** were randomized to receive either carboplatin or docetaxel. In the
unselected TNBC population, there was no evidence to support the superiority of either agent. However, in patients with germline mutations in BRCA1 or BRCA2, carboplatin was associated with a higher response rate [63]. Of note, 55% (16/29) of patients with germline mutations in BRCA1 or BRCA2 in this study had TNBC. More recently, a press release reported that the OlympiAD trial, which randomized patients harboring germline mutations of BRCA1 or BRCA2 with HER2-negative metastatic breast cancer to receive either olaparib or physician’s choice chemotherapy, demonstrated that patients receiving olaparib benefited from a statistically significant and clinically meaningful improvement in PFS. We anticipate that full details of the results will be released soon, further enhancing our ability to make therapeutic decisions for TNBC patients who harbor germline BRCA1 or BRCA2 mutations.

MOLECULAR CLASSIFICATION AND POTENTIAL TREATMENT STRATEGIES

It is hoped that the identification of TNBC subtypes will lead to therapeutic advances in the treatment of TNBC, as each subtype has molecular aberrations potentially targetable with existing FDA-approved drugs or agents currently under development (Table 2). For example, identification of the BL1 subset and its dependence on the DNA repair pathway suggests that future studies targeting the BL1 subset could exploit this dependence through the use of DNA-damaging agents such as platinum compounds and/or PARP inhibitors to improve therapeutic efficacy compared with treatment of unselected TNBC patients. Also, data from early phase trials are suggesting that targeted therapy such as androgen antagonists for AR-positive TNBC [56, 57] or mTOR inhibition in mesenchymal/metaplastic TNBC [51] may be viable options for the treatment of specific subsets of TNBC. Lastly, several treatment strategies that could potentially prove to be effective in treating the additional TNBC subtypes are currently under investigation, including the use of drugs targeting the EMT pathway and cancer stem cells, as well as immune-directed therapies.

THE WAY FORWARD

At the present moment, classifying TNBCs into molecular subtypes based on gene expression profiling remains experimental. The difficulty in applying large-scale gene expression data to clinical practice is in part due to the large number of genes involved, which invariably results in overfitting of data due to the inclusion of genes that have little or no impact on outcome, resulting in a less than ideal performance when applied in real life. Building on earlier work by Lehmann et al., Ring et al. developed a new classification model based on 101 genes using the same gene expression data sets [64]. There was considerable agreement between the two models, and a commercial assay is being developed based on this algorithm with additional studies being planned to compare the performance of both models as predictors of prognosis and response to therapy [64].

However, creating a clinically relevant classification system for TNBC is only the first step. As our understanding of the underlying biology improves, it is highly conceivable that multiple rare and inherently different subtypes of TNBC will be identified in the future and the only way to design adequately powered clinical trials for each distinct subtype would be through greater interinstitutional collaboration.

CONCLUSION

The limited success of targeted therapeutics in TNBCs can be attributed in part to molecular heterogeneity within the “catch all” diagnosis of TNBC. Until recently, most clinical trials of TNBCs have enrolled unselected populations of patients with TNBC, which results in a dilution of drug effect. Although gene expression profiling has provided much insight into the underlying biology and heterogeneity of TNBCs, subtype “calls” are very much affected by the bioinformatic methods used [10] and there is currently no consensus on the optimal way of stratifying TNBCs. In addition, the use of large numbers of genes in predictive model development has often times led to overfitting, limiting reproducibility in the real world. Although multiple methods of partitioning TNBCs based on gene expression profiles have been proposed, similarities exist between the subtypes defined by each method, suggesting that we may soon adopt a uniform method of classifying TNBCs. It is imperative to continue incorporating gene expression profiling into clinical trials of targeted therapies in TNBCs, as the data generated will allow us to retrospectively match response with patterns of gene expression, thereby helping to inform the design of future prospective studies.

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Final approval of manuscript: Clinton Yam, Sendurai A. Mani, Stacy L. Moulder

DISCLOSURES

The authors indicated no financial relationships.

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For Further Reading:

Implications for Practice:
This study pooled centrally reviewed hormone receptor (HR) and HER2 data and individual gene expression and intrinsic subtyping from three cooperative group trials. The results indicated that the optimal cut point for defining triple-negative breast cancer, if the goal is to enrich for basal-like biology, is to adopt the guideline of <1% staining. Tumors with borderline HR expression are highly biologically heterogeneous, which raises the question of whether these tumors should be considered indeterminate. A proportion of clinically defined HER2-negative tumors were defined as molecular HER2-enriched subtype; however, whether they are suitable for anti-HER2 therapy needs to be determined.