A Targetable Androgen Receptor–Positive Breast Cancer Subtype Hidden Among the Triple-Negative Cancers

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Objective.—To review and present recent literature along with our own experience regarding the clinical and morphologic characteristics and the prevalence of androgen receptor (AR) expression in TNBC, and to discuss the potential use of AR as a therapeutic target for AR+ TNBC.

Data Sources.—Data sources are published articles from peer-reviewed journals in PubMed (US National Library of Medicine).

Conclusions.—AR is the most commonly expressed hormone receptor among all breast carcinomas, with a prevalence of 25% to 75% among TNBCs. Therefore, we strongly support the routine assessment of AR in TNBC, and preferably in all breast carcinomas.


Breast cancer is the most common malignancy among women in the United States, with an estimated 232 340 new cases and 39 620 deaths in 2013.1 There has been a steady improvement in patient survival in recent decades as a result of a combination of early detection due to advances in mammographic screening2 and adjunct systemic chemotherapy.3 Identification of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) as predictive and prognostic markers in breast carcinoma has been one of the major achievements of the past several decades. Expression of these markers in breast carcinomas has led to targeted therapy with tamoxifen, aromatase inhibitors, and trastuzumab, improving prognosis and outcome while reducing the undesirable side effects of nonspecific chemotherapy. It is well known, however, that breast cancer is highly heterogeneous at the morphologic, functional, and molecular levels. Approximately 10% to 24% of breast cancers lack ER, PR (by immunohistochemistry), and HER2 expression (by immunohistochemistry and/or gene amplification).4–7; these tumors qualify as triple-negative breast carcinomas (TNBCs). It is important to note that the prevalence of TNBC depends on the threshold for positivity used in the assessment of these biomarkers in various studies. In the latest College of American Pathologists/American Society of Clinical Oncology guidelines, the threshold used for ER and PR has been reduced to a mere 1% of the invasive cancer cells.8 In addition, the threshold for HER2 positivity has been reduced from 30% to 10% when using the immunohistochemical approach, and for in situ hybridization the Her2/CEP17 ratio for Her2 gene amplification is now >2 (reduced from >2.2), and a HER2 copy number of >6 signals per cell is also sufficient for Her2 positivity.9 Patients with TNBC, lacking an option for targeted therapy, are excluded from the benefits of this approach. Clearly, the identification of novel targets for therapy among the TNBCs is of major interest, even though the current American Society of Clinical Oncology/College of American Pathologists guidelines will eliminate some tumors that would have qualified as TNBC based on the prior guidelines for ER, PR, and HER2 assessment.

Although early gene expression profiling–based studies suggested that as a group TNBCs are biologically more aggressive, this is not the case across the diverse spectrum of these tumors. As an example, adenoid cystic carcinomas are triple negative but are among the least aggressive breast cancers. Also, medullary carcinomas are triple negative with high-grade morphology and occur among women with or without BRCA germ line mutations, but they are not highly aggressive when presenting as low-stage, node-negative tumors. Among carcinomas of special types, the squamous and myoepithelial carcinomas are epidermal growth factor receptor (EGFR) positive and could potentially benefit from...
EGFR-targeted therapy. About 25% to 75% of TNBCs show androgen receptor (AR) expression; many, but not all, of these tumors are apocrine carcinomas. It is this latter group (AR+ TNBCs) that is of special interest in this review for a potential benefit from AR-targeted therapies.

**CLINICAL FEATURES OF TNBC**

Triple-negative breast carcinomas are 2- to 3-fold more common among black women compared with white women. In a study of 1149 invasive breast cancer patients (518 African American, 631 white), basal-like (ER-, PR-, HER2-, EGFR+, or CK5/6+) breast carcinomas accounted for 11% of carcinomas among white patients, but 22% of breast carcinomas among African American patients. Furthermore, the percentage of basal-like tumors was highest (29%) among premenopausal African American women, compared with 17% among postmenopausal African American patients, and 15% and 10% among premenopausal and postmenopausal white patients, respectively. Interestingly, however, mortality for patients with basal-like breast cancer (BLBC) has been reported as being higher among white patients (hazard ratio, 2.0; 95% confidence interval, 1.2–3.4) than African American patients (hazard ratio, 1.5; 95% confidence interval, 1.0–2.4) in some studies, whereas others have noted higher mortality for African Americans even after adjusting for treatment and comorbidities. Other studies have also noted a higher frequency of basal-like carcinomas among young African Americans and younger women in Africa compared with European American, European, and Asian women. Aside from being younger, women with TNBC have both modifiable and nonmodifiable risk factors that include earlier age at menarche and at first pregnancy, increased parity, decreased breast-feeding, higher body mass index, and lower socioeconomic status, based on multiple population-based studies.

Among hereditary breast carcinomas, about three-quarters of those with germ line BRCA1 mutations are basal-like and triple negative by immunohistochemistry and microarray. Therefore, BRCA1 mutation could be considered a risk factor for BLBC and TNBC.

Triple-negative breast carcinomas show a higher risk of recurrence and death from disease in the first 3 to 5 years after diagnosis.

**MORPHOLOGIC VARIANTS OF TNBC**

Included among TNBCs are a variety of morphologic and molecular subtypes. A high proportion (74%–83%) of TNBCs have an infiltrating duct, not otherwise specified morphology. Other variants of TNBC include medullary carcinoma, apocrine carcinoma, adenoid cystic carcinoma, carcinomas arising in microglandular adenosis, myoepithelial carcinomas, and the spectrum of metaplastic carcinomas. Among these, apocrine carcinomas are notable because they have AR expression in more than 90% of cases; this group may benefit from therapies targeted at the AR. Also, a small proportion of the triple-negative, not otherwise specified-type infiltrating duct carcinomas are AR positive (Figure 1) and may also benefit from AR-targeted therapies. Adenoid cystic carcinomas are very indolent and low-grade cancers with no known case that has had axillary node metastases; these tumors probably would not require any additional therapies following surgical excision. Myoepithelial and squamous cell carcinomas of the breast are generally EGFR positive; EGFR could be explored as a therapeutic target for these tumors. Carcinomas with osseous or chondroid differentiation, carcinomas arising from microglandular adenosis are developmentally complex lesions that require substantially more assessment at the molecular level for a better understanding of their evolution and identification of potential targets for therapy.

**MOLECULAR FEATURES OF TNBC**

It is widely recognized that TNBC reflects a highly heterogeneous group. Molecular subclassification of TNBC has resulted in identification of several points of interest for further exploration and potential therapeutic targets. These include the DNA damage response, angiogenesis, epithelial mesenchymal transition, and immune deregulation. Although some of these alterations would also apply to non-TNBCs, a better insight into the epithelial mesenchymal transition in particular would be highly relevant to the carcinomas with chondroid and/or osseous differentiation as well as carcinosarcomas. Further exploration of the functional pathways in the newly added intrinsic subtype category of claudin-low tumors—being highly enriched in the epithelial-to-mesenchymal transition markers, immune response genes, and cancer stem cell–like features—could provide important information for management of these rare and therapeutically challenging tumors.

About 20% of the basal-like TNBCs have been found to have either germ line or somatic BRCA1 or BRCA2 dysfunction. The development of poly (ADP-ribose) polymerase (PARP) inhibitors for management of TNBC in BRCA1/2 mutation carriers is an example of such exploratory efforts.

Using gene expression profiling, BLBCs are often, but not always, triple negative. Recent studies using the PAM50 intrinsic subtypes have reported a high proportion (72%, 79%, and 86%) of TNBCs qualifying as basal type. In the study where 86% of 252 TNBCs were basal-like, 7.1% qualified as HER2 enriched, 4.8% as “normal-like,” and 2% as luminal A intrinsic subtype, emphasizing the heterogeneity of this category.

**CURRENT AND FUTURE THERAPIES**

Given the lack of specific targets for therapy, most TNBCs are treated with relatively nonspecific cytotoxic agents. Patients with TNBC were found to benefit from the addition of paclitaxel to doxorubicin and cyclophosphamide in a retrospective evaluation of CALGB 9344. In a neoadjuvant setting, TNBCs appear to be sensitive to anthracycline/taxane-based regimens, based on the high frequency of pathologic complete response. When patients do not achieve a pathologic complete response, however, the likelihood of relapse is higher compared with patients with hormone receptor–positive cancers. Use of gene expression analysis to identify a panel predictive of response or lack of response to various chemotherapeutic combinations could help identify those who may or may not benefit from such therapies.

Among breast carcinomas, EGFR is more commonly expressed in TNBC and BLBC. EGFR has been explored as a potential target for therapy based on its expression in some variants of TNBC and the demonstration of EGFR dependence for growth and proliferation on BLBC cell lines. A randomized phase 2 trial, evaluated the role of EGFR inhibition in metastatic TNBC. Eligible
women in this study received the anti-EGFR monoclonal antibody cetuximab with carboplatin or received cetuximab alone with a planned crossover to carboplatin at disease progression. Although cetuximab alone was basically ineffective and this treatment arm was closed early, response to cetuximab combined with carboplatin was 17%, with clinical benefit evident in 29% of pretreated patients. In another study, irinotecan plus carboplatin was found to be modestly more effective when combined with cetuximab in TNBC; the response rate increased from 30% to 49% with the addition of cetuximab.

BRCA1/2 mutation/dysfunction results in failure of or defects in the repair of DNA double-strand breaks by homologous recombination; ultimately, this results in...
play a role in the pathogenesis of breast carcinoma and increasing. Recent and emerging data suggest that AR may and may be associated with variable response with a variety of other targets vascular endothelial growth factor. In one of the trials (BEATRICE), patients with operable TNBC are randomized to receive standard chemotherapy with or without 1 year of adjuvant bevacuzumab.49 Angiogenesis inhibitors; the latter are for those triple-negative carcinomas associated with BRCA dysfunction. Novel targets being explored include EGFR and AR.

### Characteristics of Triple-Negative Breast Carcinoma (TNBC)

1. TNBC is defined by its lack of ER, PR, and HER2.
2. TNBC is heterogeneous at the morphologic, immunohistochemical (with additional biomarkers beyond ER, PR, and HER2), and molecular levels.
3. TNBC shares negativity for ER, PR, and HER2 with BLBCs, but the latter also express basal cytokeratins (CK5/6 and CK14/17) and often, but not always, EGFR.
4. Young age at diagnosis, early relapse and metastases, and poor prognosis are more common among TNBC compared with other types of breast carcinoma.
5. Breast cancers developing in patients with BRCA1 mutations or dysfunction are often, but not always, of the TNBC and BLBC subtypes.
6. Approximately 25% to 75% of TNBCs are AR positive; not all of these are apocrine carcinomas.
7. Therapies under investigation include inhibitors of angiogenesis and poly (ADP-ribose) polymerase inhibitors; the latter are for those triple-negative carcinomas associated with BRCA dysfunction. Novel targets being explored include EGFR and AR.

Abbreviations: AR, androgen receptor; BLBC, basallike breast carcinoma; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

genomic instability. In the presence of dysfunctional BRCA, DNA repair requires the PARP enzyme; aside from germ line mutations, BRCA protein expression may be reduced by other alterations (ie, promoter methylation or loss of heterozygosity) in the BRCA pathway. A family of nuclear enzymes, PARP is crucial in the detection and repair of DNA damage. Also critical in cell proliferation, PARP is upregulated in a variety of cancers, including TNBC and carcinomas associated with BRCA1 and BRCA2. Because loss of BRCA1 and 2 results in increased dependence on PARP for DNA repair, BRCA1- and BRCA2-associated cancers are particularly likely to have sensitivity to PARP inhibitors. Because 1 in 5 patients (20%) with BLBC have either germ line or somatic BRCA1 and BRCA2 dysfunction, these patients could potentially benefit from therapy with PARP inhibitors.41 Poly (ADP-ribose) polymerase inhibitors may also enhance the activity of other DNA–damaging agents, including both cytotoxic and radiation therapy.31–55 The PARP inhibitor olaparib in combination with various cytotoxic agents is being explored in TNBC.

Also under investigation for efficacy in patients with TNBC is the angiogenesis inhibitor bevacuzumab, which targets vascular endothelial growth factor. In one of the trials (BEATRICE), patients with operable TNBC are randomized to receive standard chemotherapy with or without 1 year of adjuvant bevacuzumab.49 Angiogenesis inhibitors are not particularly specific for TNBC, however, and may be associated with variable response with a variety of other carcinomas as well.

### AR+, TRIPLE-NEGATIVE BREAST CARCINOMA

Along with increasing interest in the discovery of novel therapeutic targets for the highly heterogeneous population of breast cancers,54–57 interest in AR as a target is also increasing. Recent and emerging data suggest that AR may play a role in the pathogenesis of breast carcinoma and could be considered a potential target for therapy, particularly in TNBC.

Frequently coex pressed with ER, PR, and/or HER2, AR is the most commonly expressed (47%–90%) receptor among all types of breast cancer,13,17,57,58 with a frequency of 10% to 75% among TNBC cases.59,60 Moinfar et al60 found AR expression in 90% of grade 1 invasive breast cancers compared with 47% of grade 3 invasive breast carcinomas and concluded that AR is the most frequently expressed marker even among high-grade breast carcinomas. Although the reported expression rate for AR has varied widely—probably because of different cutoff points13–17,59,62 used in various studies—it has been suggested that its expression in TNBC has prognostic value.57,60

Using the 1% criterion for ER, PR, and AR positivity, along with a 30% threshold for HER2 immunohistochemical evaluation, a recent study of 325 invasive breast cancers found 21 cases (6.5%) that were only AR positive and 33 that were AR and HER2 positive.64 Interestingly, even among BRCA1-mutated breast cancers, about 1 in 5 tumors that are ER− and PR− express AR.65 In one recent phase 2 study of 424 metastatic breast carcinomas exploring the benefits of bicalutamide (an androgen inhibitor) in AR+, ER−, and PR− tumors, AR was expressed in 12% of the ER/PR-negative breast cancers.66 This study showed minimal toxicity and efficacy for androgen blockade in a select group of patients.66

In a recent review of 400 cases from our own institution using 1% for positivity for ER, PR, and AR, along with 30% positivity by immunohistochemistry or a HER2/CEP17 ratio of ≥2.2 for HER2, AR was coexpressed in 87.8% of our 400 cases, it was the sole receptor expressed in 4.5% of the cases, it was the only steroid receptor expressed in combination with HER2 in another 14 (3.5%) of the tumors, and it was the only receptor expressed in 36% of the 50 TNBCs. This implies that about 8% of all tumors and 36% of TNBCs could potentially benefit from using AR as a target for therapy in the adjuvant or neoadjuvant setting. Also, it seems logical to consider AR as an alternate target when ER+ and/or HER2+ carcinomas become resistant to tamoxifen, aromatase inhibitors, or trastuzumab. The characteristic features of TNBC are shown in the Table.

### COMMENT

Although AR+ TNBCs constitute a relatively small proportion of all breast carcinomas (accounting for only 1.2% to a maximum of 4.5% of all breast cancers), considering the estimated 232 000 breast carcinomas that developed in US women in 2013, this would yield a minimum of 2784 to a maximum of 10 440 patients17,67,68 who could potentially benefit from some form of AR-targeted therapy.

It is important to emphasize that AR is expressed in the normal epithelial cells of the terminal duct lobular units and those of the larger ducts, just as ER and PR are expressed. Some of the normal epithelial cells probably coexpress all three hormones, whereas others may express only one or two of these hormones. Any of these cells could be the source of a neoplastic proliferation resulting in cancers that express one or all of these biomarkers, but neoplastic cells may gain or lose expression of any of these markers. However, metaplastic apocrine cells in the breast, whether benign and nonproliferative or malignant, are generally AR positive (Figure 2). Furthermore, either the normal epithelial cells that are only AR positive constitute the source of metaplastic apocrine cells—the most common metastatic
change in the breast—or the metastatic apocrine cells may develop directly from a stem cell population. As a consequence, AR+ TNBCs developing from the nonapocrine epithelial cells that are only AR+, and those that develop directly from metastatic apocrine cells or stem cells may show totally different responses to therapies directed at AR. Exploration of possible diverse mechanisms for AR positivity could help explain why some solely AR-positive tumors respond or do not respond to antiandrogen therapy. Interestingly, apocrine cells do have the messenger RNA for ER, even though they do not express the protein.69

Finally, because in the current approach to immunohistochemical testing and interpretation, the existence and/or the reliability of ER+/PR- tumors has been questioned,70–72 and in at least one study, in ER-positive disease the PR value may not be predictive of who would respond to tamoxifen,73 one could question why we continue to assess PR status in breast carcinomas when so many other factors can provide us with the same information provided by PR status; the resources could certainly be saved and probably better allocated.70

Although we strongly advocate routine assessment of AR for at least TNBC and preferably for all breast carcinomas, additional studies are needed to determine the optimal threshold for considering a tumor as AR positive by immunohistochemistry and to better define the value of AR as both a prognostic and a predictive factor. When AR and HER2 are the only positive markers, it would be important to establish how therapies for the two targets may interact. When AR is the only target, then it would be worthwhile to determine whether the tumor’s cell (apocrine or non-apocrine) type has any impact on response to various therapies and to define any and all side effects.

References


