Role of the Androgen Receptor in Triple-Negative Breast Cancer

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Abstract: Triple-negative breast cancer (TNBC) is an aggressive disease with outcomes inferior to those of other breast cancer subtypes. No targeted therapies are currently approved for TNBC, and newer treatment approaches are critically needed. It is increasingly recognized that TNBC is a heterogeneous disease, and the role of androgen signaling in a subset of TNBC is emerging. Although the degree of androgen receptor (AR) expression in TNBC varies widely depending on the assay methodology, cutoff for positivity, and patient population, existing evidence suggests an association between a higher level of AR expression and improved outcomes. Despite lower pathologic complete response (pCR) rates with neoadjuvant therapy, patients with AR-dependent TNBCs have a better prognosis than those with TNBCs that are not AR-dependent. Furthermore, gene expression profiling has been used to identify a luminal androgen receptor subtype of TNBC that is dependent on AR signaling. Early clinical studies investigating agents targeting AR in advanced TNBC have produced promising results. We review herein the literature on the biology of AR in breast cancer and its prognostic and predictive role in TNBC, and we describe the results of early clinical trials with antiandrogens in this population. We also present our vision of the future development of newer therapeutic strategies in AR-dependent TNBC.

Keywords
AR, androgen receptor, bicalutamide, enzalutamide, TNBC, triple-negative breast cancer

Introduction

Breast cancer is the most common cancer in women worldwide and remains an important global health issue.[1] Breast cancer is diagnosed in 1 of every 8 US women during her lifetime.[2] The American Cancer Society estimated that 234,190 new cases of invasive breast cancer would be diagnosed in the United States in 2015, with 40,730 deaths.[3]

Triple-negative breast cancer (TNBC), which lacks expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 (HER2), accounts for approximately 15% of cases of breast cancer. TNBC is associated with African American or Hispanic race, a younger age and advanced stage at diagnosis, a high mitotic index, and *BRCA1* mutations.[4-8] Owing to the absence of any targeted therapies, cytotoxic chemotherapy remains the mainstay of medical treatment for TNBC, but outcomes are poor compared with those for other subtypes.[9,10] The median survival of women with advanced TNBC remains a dismal 13 months.[10] Improved understanding of this disease is urgently required to advance our treatment approaches.

Over the last decade, gene expression profiling has been used to classify invasive breast cancers into biologically and clinically distinct subtypes. Based on gene expression classification, the majority of TNBC cases are of the basal-like subtype.[11] Phenotypically, the basal-like subtype is characterized by a high histologic grade, high mitotic indices, early disease recurrence, and poor outcomes.[12-14] Thus, the terms *TNBC* and *basal-like breast cancer* often are used interchangeably.

However, researchers are increasingly recognizing that TNBC is a heterogeneous disease that encompasses distinct intrinsic molecular subtypes. Lehmann and colleagues were one of the first groups to use gene expression profiling to subclassify TNBC. They identified 6 distinct subtypes: (1) basal-like 1 (17%), which is characterized by a higher expression of cell cycle, DNA repair, and proliferation genes; (2) basal-like 2 (7%), which is characterized by the upregulation of genes in the growth factor signaling pathway; (3) immunomodulatory (18%), which is enriched for immune cell processes; (4) mesenchymal (17%) and mesenchymal stem–like (14%), which are enriched for epithelial-mesenchymal transition and growth factor pathways; (5) unstable (14%); and (6) luminal androgen receptor (LAR, 12%).[15,16] Similarly, Jézéquel and colleagues used gene expression profiling to identify 3 distinct subtypes of TNBC: an LAR subtype, a basal-like subtype with a low immune response, and a basal-like subtype enriched with a high immune response.[17] Subsequently, other groups validated the LAR subtype as a distinct subtype of TNBC.[18]

The LAR subtype is enriched for hormonally regulated pathways and is dependent on androgen receptor (AR) signaling.[15] Although AR can be expressed in multiple molecular subtypes of TNBC, the LAR subtype has the highest level of AR expression.[19] Distinct from unselected TNBC, the LAR subtype is predominantly subclassified in the nonbasal subgroup and represents a novel subtype of TNBC with a distinct prognosis that offers an opportunity to develop targeted therapeutics.[16] We focus here on the prognostic and predictive role of AR in TNBC, with a particular emphasis on its potential clinical implications.

Androgen Receptor Expression: Biology in Breast Cancer

AR is a member of the nuclear steroid hormone receptor family, which also includes ER and PR. Steroid hormone receptors are critical components of signaling pathways and play a crucial role as transcription factors regulating gene expression. Although ER and PR are widely recognized for their prognostic and predictive roles in breast cancer, the biological role of AR in breast cancer is still emerging.

Androgens, including testosterone and dihydrotestosterone, are involved in the function of multiple female organs, including the reproductive tract, bones, kidneys, and muscles. They act either indirectly, as prohormones of estradiol, or directly, by binding to AR.[20,21] The binding of circulating androgens to AR leads to translocation of the receptor to the nucleus, binding to target genes, and transcriptional activation.[22] Preclinical studies have shown that the androgen signaling pathway plays a critical role in the development of normal and malignant breast tissue, with animal models implicating androgen signaling in the progression of breast carcinoma.[23,24] Epidemiologic studies have suggested that increased levels of circulating androgens are associated with an increased risk for breast cancer, primarily ER/PR-positive breast cancers.[25]

AR is expressed in 2 types of mammary epithelial cells. It is most uniformly and diffusely expressed in metaplastic apocrine cells, which are a component of fibrocystic changes. The majority of these apocrine cells lack expression of ER and PR.[26] AR is also expressed in 5% to 30% of luminal epithelial cells, where it is commonly coexpressed with ER/PR. Tumors arising from these 2 different cell populations may share expression of AR but are morphologically distinct.[27] Furthermore, the responses to targeting AR therapeutically can differ based on the origin of a tumor in apocrine cells vs luminal cells.

Although growing evidence supports the role of androgens and AR in the pathogenesis of breast cancer, the role of the AR pathway in TNBC remains uncertain.[28-30] AR is expressed in 70% to 90% of primary breast cancers, a frequency that is comparable to or higher than that of either ER or PR.[30-32] Significant variability exists in the reported literature regarding the frequency of AR expression in TNBC, however, with a wide range of 6.6% to 75%.[29,33-38] This heterogeneity primarily results from variability among reported studies in terms of the number of patients included and the cutoff used for AR positivity (≥1% or >10%). The source of the primary antibody

and the methodology of testing are other reasons for the variability among different studies. Another possible reason for the variability could be the confounding effects of patient selection in prospective studies. In one of the largest systematic reviews, which included 7693 breast cancers among 19 studies, AR expression was 74.8% in ER-positive tumors and 31.8% in ER-negative tumors.[32]

Despite the establishment of AR expression, the function of AR in breast cancer is still being elucidated. Several preclinical studies have implicated AR as a potential tumor suppressor in ER-positive breast cancer, with antiproliferative effects due to the cross talk between the steroid receptor signaling pathways.[39] Androgens such as testosterone and dihydrotestosterone can have either an inhibitory or a stimulatory effect on breast cancer cell lines depending on the coexpression of other steroid hormone receptors and the presence or absence of breast adipose tissue fibroblasts (BAFs).[40] Testosterone induces cell proliferation in ER-positive MCF7 and T47D cell lines, but not in triple-negative MDA-MB-231 tumor cells, in the presence of BAFs. This has been explained by the high level of expression of aromatase, which converts androgens to estrogens in BAFs, followed by ER-mediated cell proliferation.[40,41] In contrast, dihydrotestosterone causes a suppression of cell proliferation in both ER-positive MCF7 and T47D cell lines as well as in ER-negative MDA-MB-231 cell lines because dihydrotestosterone is not a substrate for aromatase.[40] Doane and colleagues demonstrated a proliferative response to androgens in the ER-negative MDA-MB-453 cell line in an AR-dependent and ER-independent manner, suggesting the potential for therapeutic strategies targeting the androgen signaling pathway.[42] Cochrane and colleagues demonstrated inhibition of both dihydrotestosterone-mediated and estradiol-mediated proliferation of ER-positive and AR-positive breast cancer cells by antiandrogens.[43] With conflicting preclinical evidence, the precise biological role of AR in TNBC remains to be elucidated but is worth pursuing further.

Prognostic Significance of Androgen Receptor Expression in TNBC

Several analyses based on unselected breast cancer cohorts have shown AR to be related to ER and PR expression and to be a marker of low-grade, well-differentiated disease.[30,39,44-46] Similarly, in TNBC tumors, a number of studies have shown that positivity by AR immunostaining is a favorable prognostic factor and associated with a lower clinical stage, lower histologic grade, and lower mitotic score.[28,29,38,47,48] Both Rakha and colleagues and Sutton and colleagues have shown that the absence of AR expression is associated with an increased risk for recurrence and distant metastasis in lymph node-positive TNBCs.[49,50] Similarly, Luo and colleagues have shown AR expression to be correlated with higher 5-year disease-free survival (DFS) and overall survival (OS) in patients with TNBC.[48] Gasparini and colleagues evaluated AR expression and its association with clinical (race, survival) and pathologic (basal/nonbasal subtype, stage, grade) factors and found AR expression to be inversely correlated with tumor grade and associated with better OS in nonbasal TNBC.[36] Similarly, Qu and colleagues retrospectively analyzed AR expression in early breast cancer and found AR to be associated with improved DFS in TNBC. However, in terms of OS, their analysis showed AR to be associated with improved OS in ER-positive disease but not in TNBC.[51] Pistelli and colleagues have shown AR expression in TNBC to be inversely correlated with a higher level of Ki-67 and lymphovascular invasion; no association was seen with DFS or OS.[52]

However, this favorable prognostic significance of AR is not uniform across the literature. Controversy exists, with discordant findings among certain studies. Hu and colleagues have shown AR expression to be associated with increased mortality among women with ER-negative and TNBC tumors.[47] Similarly, Park and coinvestigators have shown a trend toward poorer outcomes in AR-positive, ER-negative breast cancers.[53] Choi and colleagues have reported AR expression to be a significant predictor of worse DFS and OS in TNBC without lymph node involvement. However, they could not identify AR as a prognostic marker in patients with TNBC and lymph node metastasis.[54] McGhan and colleagues have found AR expression to be associated with lymph node metastasis in TNBC.[34]

This discrepancy among studies could be due to the overlap between AR and molecular apocrine signatures in TNBC tumors. Molecular apocrine tumors are a distinct subset of TNBCs characterized by AR expression and AR pathway activation. Gene expression studies have shown molecular apocrine tumors to have a paradoxical expression of genes typically expressed in ER-positive breast cancers as a consequence of AR-driven transcription of the ER pathway.[42,55,56] Molecular apocrine tumors have a poorer prognosis and thus could negate the positive prognostic influence of AR.[57] In addition, the prognostic discrepancy could be due to variations in sample sizes, source and sensitivity of the primary antibody used to detect AR, adjuvant treatments, and length of follow-up among studies. Taken together, these studies show that further investigation to elucidate the prognostic implications of AR in TNBC is required. Routine AR evaluation by immunohistochemistry (IHC) in TNBC could provide further insight in this direction.

Response to Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer

Neoadjuvant chemotherapy before surgical resection is increasingly being used in TNBC. The NSABP (National Surgical Adjuvant Breast and Bowel Project) Protocols B-18 and B-27 have shown the neoadjuvant approach to be equivalent to adjuvant therapy in terms of survival benefit.[58] Principally, neoadjuvant chemotherapy provides an opportunity to downstage bulky disease and make breast-conserving surgery feasible. The neoadjuvant approach also allows the response to treatment to be assessed. Several studies have shown an association of DFS and OS with the clinical and pathologic tumor response to neoadjuvant chemotherapy in TNBC.[59-61] Approximately one-third of patients who have TNBC treated with anthracycline and taxane neoadjuvant chemotherapy achieve a pathologic complete response (pCR).[62,63] Although the rate of pCR is significantly higher in TNBC than in ER-positive breast cancer, patients who do not attain a pCR and have residual invasive disease after neoadjuvant chemotherapy have a significantly higher risk for recurrence and death.[60] Prospective studies and a meta-analysis have shown that the 3-year DFS in patients with TNBC who attain a pCR is approximately 90%, compared with approximately 60% in patients who have residual disease after neoadjuvant chemotherapy. Similar results are seen for OS.[60,64] Owing to the poor prognosis of those with residual TNBC after neoadjuvant chemotherapy, multiple clinical trials are currently exploring novel agents in this niche.

In order to explore the heterogeneous response of TNBC to neoadjuvant chemotherapy, Masuda and colleagues evaluated clinical outcomes in 130 patients based on subtypes of TNBC.[16] They found that patients with the basal-like 1 subtype had the highest pCR rate (52%). In contrast, those with the LAR subtype had one of the lowest pCR rates (10%). However, despite their low pCR rate, OS was better in patients with the LAR subtype.[16] Another unique characteristic of the LAR subtype was that 75% of cases of distant metastasis occurred more than 3 years after diagnosis. Others have also evaluated the prognostic significance of AR for neoadjuvant chemotherapy. Loibl and colleagues evaluated AR expression by IHC in patients who had primary breast cancer treated with neoadjuvant docetaxel, doxorubicin, and cyclophosphamide (TAC) in the GeparTrio trial from the German Breast Group; they found no significant difference between the pCR rates of patients with AR-positive TNBC tumors (29.2%) and those with AR-negative TNBC tumors (33.3%). However, the patients with AR-positive TNBC tumors had significantly better DFS (85.7% vs 65.5%, P=.05) and OS (95.2% vs 76.2%, P=.03) than the patients with AR-negative TNBC tumors.[16,61] Yu and colleagues also evaluated chemoresistant tumors with gene expression profiling in patients who had residual disease after neoadjuvant chemotherapy. They found the LAR subtype of TNBC, with a high level of expression of "luminal-like" genes, to have a relatively favorable prognosis compared with tumors expressing cancer stem cell markers.[18]

These studies indicate that AR-dependent TNBCs, defined by LAR subtype on gene expression profiling or AR expression by IHC, have a better prognosis than AR-independent TNBCs despite having a lower pCR rate. However, in the study of Loibl and colleagues, 22.5% of these patients had a recurrence at 5 years.[61] Therefore, consideration of an alternate therapy is merited to further decrease the risk for relapse in this chemoresistant population. Targeting AR offers a biologically promising strategy.

Targeting the Androgen Receptor in Advanced Triple-Negative Breast Cancer

The first clinical studies to investigate targeting AR in advanced breast cancer were performed in the 1980s. Both Perrault and colleagues and Zhao and coinvestigators examined the use of flutamide, an oral antiandrogen, in advanced breast cancer. However, these did not show any meaningful activity and therefore were not pursued further at that time.[65,66]

More recently, Gucalp and colleagues presented the first evidence regarding the clinical efficacy of targeting AR in advanced TNBC. These researchers performed a single-arm, nonrandomized, phase 2 clinical trial with bicalutamide, an oral nonsteroidal AR antagonist, in AR-positive, ER/PR-negative (0%-10%) metastatic breast cancer[67] (Table). The US Food and Drug Administration has approved bicalutamide for use in combination with luteinizing hormone–releasing hormone analogues for the treatment of metastatic prostate cancer. In this clinical trial, the majority of the patients had visceral disease and had received a median of 1 (range, 0-8) prior line of chemotherapy for metastatic disease. A total of 424 patients with ER/PR-negative disease were screened for the trial, and 51 patients (12%) were positive for AR based on the preselected criterion for AR positivity, which was defined as AR expression of greater than 10% by IHC. Only 1 patient with HER2-positive disease was enrolled. A total of 26 patients in the study received treatment with bicalutamide. Although there were no objective responses, this trial showed an intriguing clinical benefit rate (CBR) at 24 weeks of 19%, with a median progression-free survival (PFS) of 12 weeks. Bicalutamide was well tolerated, with no grade 4/5 treatment-related adverse events. This trial demonstrated the first proof of principle for the efficacy of minimally toxic androgen blockade in advanced AR-positive TNBC, and its findings have formed the basis for further clinical trials in this population.[67]

Table. Summary of Clinical Studies Targeting the Androgen Receptor in Metastatic Triple-Negative Breast Cancer

Agent	Patients, n	AR Positivity by IHC	Lines of Prior Chemotherapy, median	CBR at 24 Weeks	Median PFS
Bicalutamide[67]	26	>10%	1 (range, 0-8)	19% (95 CI, 7%-39%)	12 weeks (95% CI, 11-23)
Enzalutamide[71]	75	≥10%	1 (range, 0-5)	29% (95% CI, 20%-41%)	14.7 weeks (95% CI, 8.1-19.3)

AR, androgen receptor; CBR, clinical benefit rate; IHC, immunohistochemistry; PFS, progression-free survival.

Enzalutamide (Xtandi, Astellas/Medivation) is a novel targeted AR inhibitor that competitively binds to the ligand-binding domain of AR and inhibits AR translocation to the cell nucleus, recruitment of AR cofactors, and AR binding to DNA.[68] Achieving significant improvements in DFS and OS, enzalutamide is approved for the treatment of metastatic castration-resistant prostate cancer both before and after chemotherapy.[69,70] Preclinical data demonstrated activity in AR-positive TNBC cell lines,[43] and Traina and colleagues evaluated the efficacy of this potent antiandrogen in a single-arm, nonrandomized phase 2 clinical trial in advanced AR-positive TNBC[71] (Table). Women were prescreened for AR, and for trial eligibility, AR positivity was defined as AR expression of greater than 0% by IHC. A total of 404 patients were tested for AR positivity; 79% had AR expression of greater than 0% and 55% had AR expression of 10% or higher. A total of 118 women were enrolled in this trial, of whom more than 50% were treated in the first- or second-line setting. The results of this study showed promising CBRs of 25% at 16 weeks and 20% at 24 weeks in patients whose tumors had AR positivity greater than 0%. In 75 patients with tumors having AR positivity of at least 10%, the CBRs were further improved to 35% at 16 weeks and 29% at 24 weeks. Notably, these results included 2 complete responses and 5 partial responses. The median PFS in patients with tumors having AR positivity of at least 10% was an impressive 14.7 weeks, vs 12.6 weeks in patients with tumors having AR positivity of greater than 0% in the intent-to-treat arm. Enzalutamide was well tolerated, with the only grade 3 or higher adverse events being fatigue (5%), dyspnea (3%), nausea (1%), constipation (1%), and back pain (1%). There were no new safety signals compared with prior studies in men who had prostate cancer.[71] Tumors from responders to enzalutamide were noted to cluster within a distinct pattern of genes that included AR, leading to the development of a predictive assay that has been termed Predict-AR.[72] Patients who had tumors that were Predict-AR–positive had a CBR of 36% at 24 weeks, compared with 6% in those whose tumors were Predict-AR–negative.[71] PFS and OS in the patients with Predict-AR–positive TNBC were 16 weeks and not reached, respectively, compared with 8 weeks and 32 weeks, respectively, in those with Predict-AR–negative TNBC.[71] With this encouraging efficacy of enzalutamide seen in advanced AR-positive TNBC, further confirmatory clinical studies are being planned.

Newer Treatment Strategies on the Horizon

Several novel antiandrogenic agents are currently under investigation in AR-positive TNBC tumors. One such promising therapeutic target in AR-positive malignancies is 17,20-lyase (CY17 or CYP17), which is a key, rate-limiting enzyme in the androgen biosynthesis pathway. Orteronel (TAK-700) is a nonsteroidal, reversible, selective 17,20-lyase inhibitor with activity in castration-resistant prostate cancer.[73,74] Based on the promising activity of orteronel in targeting AR in TNBC, a phase 2 study is ongoing to evaluate its efficacy in patients with AR-positive metastatic breast cancer, with a separate cohort for AR-positive TNBC (NCT01990209).[75] Another promising agent in this arena is VT-464, a novel, second-generation, small-molecule, lyase-selective CYP17 inhibitor. A phase 1 dose-finding study of this oral agent is ongoing in AR-positive TNBC (NCT02580448).[76,77]

In addition to single-agent studies, significant interest is being shown in potential strategies combining AR antagonists with other targeted treatments (Figure). Lehmann and colleagues have found AR-positive TNBC tumors to have a higher frequency of phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha gene (*PIK3CA*) mutations compared with AR-negative tumors (40% vs 4%), and these are often associated with concurrent amplification of the *PIK3CA* locus. In cell line models and xenograft studies, combining bicalutamide with a pan–phosphoinositide 3-kinase (PI3K) inhibitor or a dual PI3K/mammalian target of rapamycin (mTOR) inhibitor has shown additive effects. This has resulted in studies assessing combinations of AR-targeted therapies with PI3K/mTOR inhibitors in advanced AR-positive TNBC tumors.[78] A phase 1b/2 clinical trial of taselisib, a PI3K inhibitor, in combination with enzalutamide in advanced TNBC is currently recruiting patients (NCT02457910).[79]

Cyclin-dependent kinases 4 and 6 (CDK4 and CDK6), activated by cyclin D, promote cell cycle entry by phosphorylating Rb (retinoblastoma) and other proteins to initiate transition from the G1 phase to the S phase.[80]

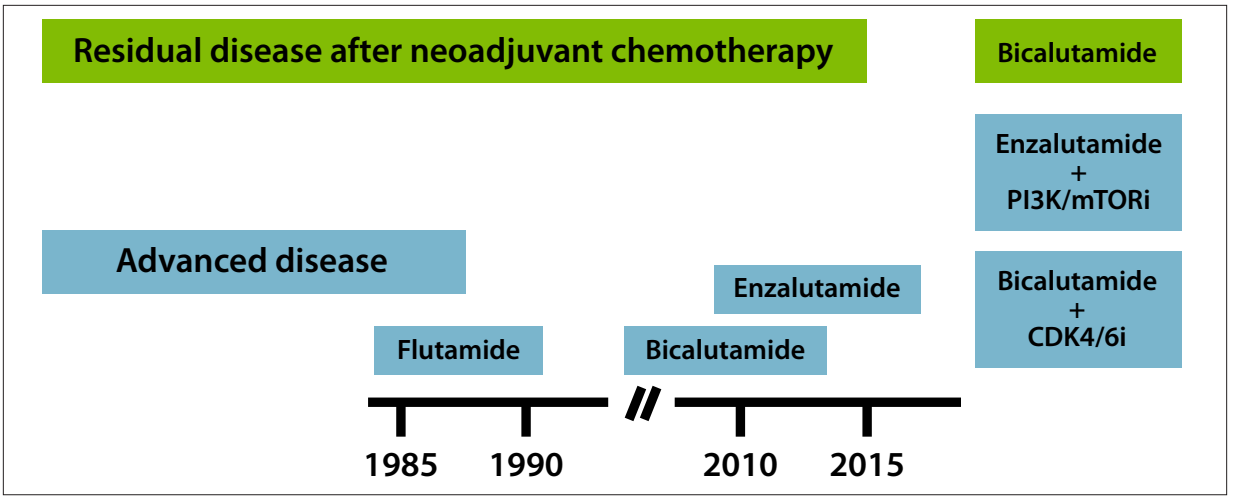

Figure. Timeline of the development of androgen receptor–targeted therapies in triple-negative breast cancer. PI3K/mTORi, phosphoinositide 3-kinase/mammalian target of rapamycin inhibitor; CDK4/6i, cyclin-dependent kinase 4/6 inhibitor.

This pathway is disrupted in many human cancers, leading to unrestrained cell proliferation.[81] After showing encouraging preclinical efficacy in breast cancer, several inhibitors of CDK4 and CDK6 are currently in varying stages of development.[82] Based on the significant improvement seen in PFS, the CDK4/6 inhibitor palbociclib (Ibrance, Pfizer) has been approved for the frontline treatment of advanced ER-positive breast cancer in combination with letrozole.[83] Recent data also support the role of palbociclib with fulvestrant (Faslodex, AstraZeneca) in patients who have had prior progression on endocrine therapy.[84]

Although TNBC has an inherently higher proliferation rate, limited activity has been seen in preclinical studies with CDK4/6 inhibitors in unselected TNBC tumors. However, more recently, the LAR subtype of TNBC has been shown to be particularly susceptible to CDK4/6 inhibition.[85] Further, in cell line and xenograft models, resistance to antiandrogen therapy has been linked to the emergence of an F876L mutation in AR that confers an antagonist-to-agonist switch driving phenotypic resistance. CDK4/6 inhibitors have been shown to effectively antagonize AR F876L function and restore sensitivity to antiandrogen therapy.[86] Although this was demonstrated in prostate cancer cell lines, the effect could be present across AR-dependent tumor types, including AR-positive TNBC. With encouraging activity of antiandrogens in AR-positive advanced TNBC, preclinical evidence of efficacy of CDK4/6 inhibitors in LAR TNBC cell lines, and preliminary evidence of the ability of CDK4/6 inhibition to overcome antiandrogen resistance, we are proposing a phase 2 single-arm study of bicalutamide in combination with ribociclib, a highly specific CDK4/6 inhibitor, in advanced AR-positive TNBC.[87] This study will provide evidence of efficacy of the combination in AR-positive TNBC, establishing a basis for subsequent randomized phase 3 studies comparing it with standard cytotoxic chemotherapy. Further, we plan an analysis to evaluate how the degree of AR positivity by IHC affects the efficacy of the combination and to identify biomarkers of response/resistance in circulating tumor cells and tumor tissue. A similar study of bicalutamide in combination with palbociclib in AR-positive metastatic breast cancer is also ongoing (NCT02605486).[88]

Because AR-dependent TNBCs are inherently chemoresistant and have a lower pCR rate with traditional neoadjuvant chemotherapy, consideration of some alternate therapy to further decrease the risk for relapse after surgery in patients without a pCR is merited.[16] Based on this hypothesis, we are conceptualizing a single-arm phase 2 clinical trial in which women with TNBC who have residual disease after neoadjuvant chemotherapy will receive adjuvant antiandrogen therapy for 1 year. The 2-year DFS will be the primary endpoint of this study. AR expression will be assessed and quantified in primary samples, samples taken after neoadjuvant chemotherapy, and metastatic samples, and resistance pathways will be evaluated in tumor samples and circulating tumor cells. This clinical trial will offer the first proof of principle for AR-targeted therapy in this distinct population.

Thus, AR identification has been a major advance in the treatment of TNBC tumors. AR represents a novel therapeutic target in TNBC, which has an otherwise inferior prognosis. Based on promising early clinical data, we anticipate that the newer, more potent antiandrogens will significantly improve outcomes and likely will be the first targeted therapy available for what to date has been an orphan disease.

Disclosures

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