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REVIEW

Harnessing a Different Dependency: How to Identify and Target Androgen Receptor-Positive Versus Quadruple-Negative Breast Cancer

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Abstract The androgen receptor (AR) is a promising therapeutic target for a subset of triple-negative breast cancers (TNBCs) in which AR is expressed. However, the mechanistic action of AR and the degree to which primary and metastatic tumors depend on AR, both before and after conventional treatment, remain to be defined. We discuss preclinical and clinical data for AR+ TNBC, the difficulties in monitoring AR protein levels, new methods for determining AR status, the influence of AR on "stemness" in the context of TNBC, the role of combined inhibition of sex steroid production and AR, and the role of AR in regulation of the immune system. Although the exact role of AR in subsets of TNBC is still being characterized, new therapies that target AR and the production of androgens may provide additional options for patients with TNBC for whom chemotherapy is currently the sole treatment option.

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Introduction

The androgen receptor (AR) is widely expressed in breast cancer (BC) [1]. It is expressed in up to half of triple-negative BC (TNBC) tumors [2], which, by definition, lack estrogen and progesterone receptors (ER and PR) as well as amplification of the human epidermal growth factor receptor 2 (HER2). Preclinical and clinical data suggest that AR is a promising therapeutic target for a subset of BC and perhaps should be the fourth receptor to be routinely examined. In this review, we discuss the roles of AR in TNBC, methods for detecting AR status, the influence of AR on "stemness" in the context of TNBC, inhibition of AR and/or sex steroid production, and AR in the immune system. Our goal is to describe the current state of research that underlies the development of novel-targeted therapy for the treatment of AR-expressing (AR+) TNBC.

The Role of Androgen Receptor Signaling in Breast Cancer

Targeted treatment for BC has historically focused on ER and HER2; however, AR is emerging as another promising therapeutic target since it is even more widely expressed in BC than ER and PR [3]. In a study of 2171 invasive BCs in women, AR protein was expressed in 77% overall, but expression varied by BC subtype, with 88% of ER+ BC, 59% of HER2+ BC, and 32% of TNBC positive for AR protein by immunohistochemistry (IHC) [1, 4]. In a study of 32 men with breast cancer, AR was expressed in 65% of all BCs and in 85% of ER+ tumors [5]. However, the mechanistic action of AR in the clinically defined BC subtypes and the manner and degree to which primary and metastatic tumors of different subtypes rely on AR, both prior to treatment and after

conventional therapy, are still actively being determined in preclinical models and clinical trials.

As with PR, AR expression has been associated with a more favorable prognosis and prolonged survival in patients with ER+ breast cancer [6–9]. The prognostic value of AR is less clear for BC tumor types that are not ER+ [10]. Although AR positivity is indicative of a more well-differentiated tumor and therefore has a better prognosis [11–13], among the TNBC subtypes, the luminal AR TNBC subtype that expresses high AR levels had a lower pathological complete response (pCR) to neoadjuvant chemotherapy than other TNBC subtypes [14]. Recent preclinical studies suggest that AR can drive growth and survival in ER+, HER2+, and TNBC cell lines [4, 15–22]. In ER+ BC, AR becomes particularly important in the context of resistance to anti-estrogen or aromatase inhibitor therapy, where tumor cells can evolve to become growth-dependent on androgens and AR under conditions of ER inhibition or estrogen deprivation [4, 17, 23–25]. A high ratio of AR:ER protein is associated with an increased risk of recurrence while on tamoxifen and decreased likelihood of disease-specific and overall survival [4]. In addition, the AR inhibitor enzalutamide blocked both androgen- and estrogen-stimulated tumor growth in AR+/ER+ BC xenograft and PDX preclinical models [4, 17]. In ER+ BC cell lines, complex interactions between AR and ER lead to transcriptional activity that affects the expression of genes involved in BC growth and survival [17].

AR is also significantly associated with HER2 amplification and promotes cell proliferation following treatment with the androgen dihydrotestosterone (DHT) or other synthetic androgens [20, 26–28]. Based on studies in the HER2-enriched BC cell line MDA-MB-453 (ER−/HER2+/AR+), AR induces an increase in HER3 via the Wnt signaling pathway to promote HER2 signaling [10, 20]. Another positive feedback loop that may feed into HER2-mediated cell proliferation exists in the AR and ERK pathways [29]. Synergistic inhibition of proliferation was observed in vitro and in vivo with combined mTOR inhibition and anti-androgens in multiple HER2+ and TNBC cell lines containing activating PIK3CA mutations [21]. Depending on the cell line, DHT induced an increase in either phosphorylated HER2 (pHER2), phosphorylated HER3 (pHER3), or both, that was attenuated by AR inhibition. Conversely, inhibition of the mTOR pathway caused an increase in total AR, pHER2, and pHER3, and these effects were abrogated by enzalutamide and seviteronel [21].

Interestingly, in a rat model of obesity-associated postmenopausal mammary carcinoma, nuclear AR was higher in tumors that progressed after ovariectomy compared to tumors that regressed. Administration of enzalutamide blocked tumor progression in rats after ovariectomy and prevented new tumor formation [30]. IL-6, which was higher in plasma of obese versus lean rats, sensitized BC cells to low levels of testosterone [30], providing an example of how obesity-associated cytokines and growth factors can affect how tumors respond to steroid hormones and hormonal therapy in all subtypes of BC.

AR regulates growth factors such as the EGFR ligand amphiregulin (AREG) in TNBC cell lines in vitro and in vivo, and AR activation and inhibition significantly affected levels of AREG [16] and other factors such as JAG1 (a ligand for Notch receptors and target of the canonical Wnt signaling pathway in progenitor cells), chitinase (CHI3L1/YKL40), and growth/differentiation factor (GDF)-15 [22]. Perhaps future studies will identify the key AR-regulated proteins most indicative of AR dependence, but these may differ with BC subtype, disease progression, and prior treatment.

Androgen Receptor Inhibition in Triple-Negative Breast Cancer

TNBC comprises approximately 15 to 20% of newly diagnosed BCs [31]. TNBC is an aggressive BC subtype with a risk of recurrence that peaks around 3 years after diagnosis [31, 32]. Because TNBC lacks the most common therapeutic targets ER, PR, and HER2, chemotherapy is the only available therapeutic option [15]. There are, as yet, no U.S. Food and Drug Administration (FDA)-approved targeted therapies available for chemoresistant TNBC disease (although the PARP [poly ADP ribose polymerase] inhibitor olaparib is anticipated to be approved for germline BRCA-mutated BC in the near future), but one avenue of current research is focused on the therapeutic inhibition of AR (Fig. 1).

Gene expression profiling has revealed four subtypes of TNBC: basal-like 1 (BL1), basal-like 2 (BL2), mesenchymal-like (ML), and luminal androgen receptor (LAR), each with distinct gene signatures [19, 33]. The LAR subtype, which expresses high AR, is of particular interest because it closely resembles the previously described molecular apocrine tumors [26, 34] and has a gene expression profile and chromatin-binding pattern similar to luminal, ER+ BC despite being ER-negative [19, 26, 34–36]. Recent studies found up to half of TNBC to be AR+ (defined as > 10% of tumor cells staining positive for AR), irrespective of TNBC subtype [37]. While the function of AR in TNBC is not well known, preclinical data suggest that AR drives tumor growth and survival, even in cells that express relatively low levels of AR. Consequently, AR is currently under investigation as a potential therapeutic target for TNBC tumors [4, 16, 19, 22, 37].

High AR and its regulation of classically ER-controlled genes make the LAR TNBC subtype more luminal (hence the designation) [19] and slower growing. A comparison of the clinical relevance of the subtype classification of TNBC reported that the BL2 and LAR subtypes had the lowest pathological complete response rates following neoadjuvant

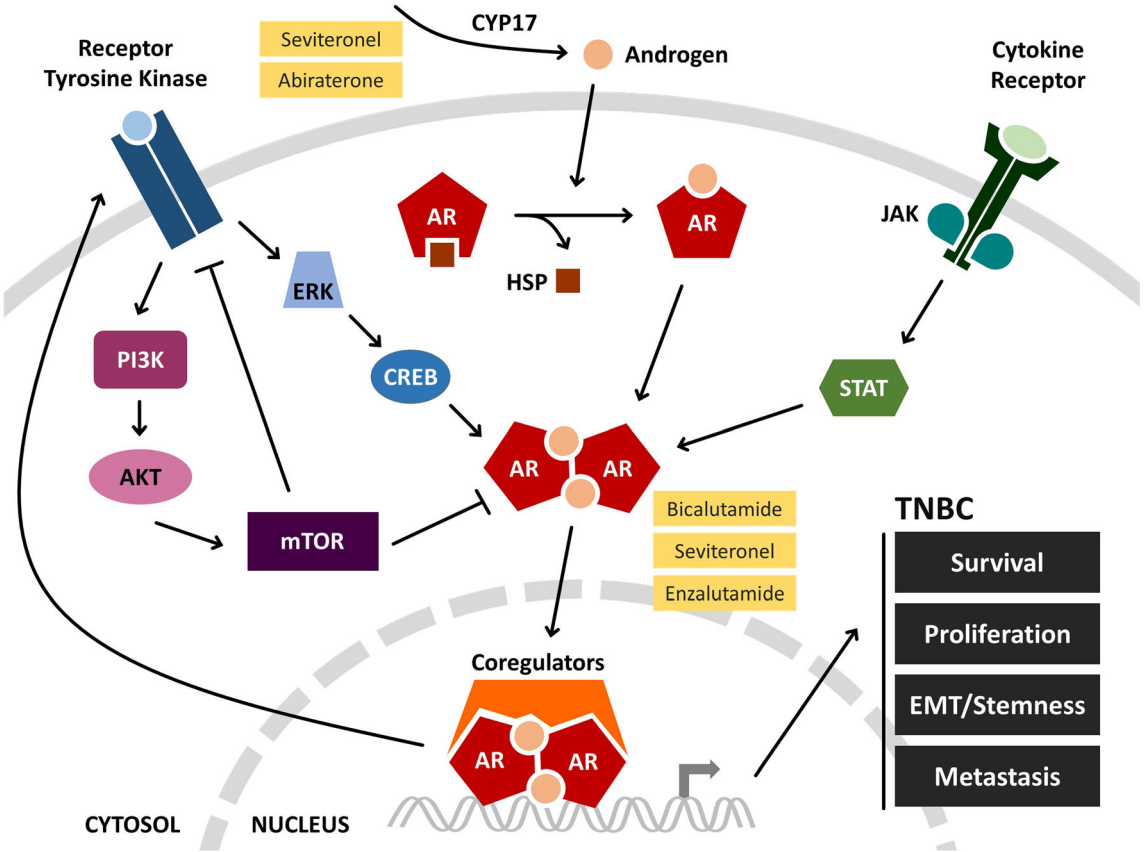

Fig. 1 AR signaling and therapeutic interventions in TNBC. Androgen synthesis is catalyzed by the enzyme CYP17. Binding of androgens to AR causes dissociation from HSP and AR dimerization. AR dimers translocate to the nucleus, associate with coregulatory proteins, and initiate transcription. In TNBC, AR signaling promotes cancer progression, and this can be blocked at various stages with pharmacological inhibitors (yellow). Cross-talk between AR and other signaling pathways is being used to establish rational drug combinations that are currently being explored for their effectiveness. Interconnecting lines are indicative of overall effects, positive or negative, on pathway activation. *AKT* protein kinase B, *AR* androgen receptor, *CREB* cAMP response element-binding protein, *CYP17* cytochrome P450 17, *EMT* epithelial-to-mesenchymal transition, *ERK* extracellular signal-regulated kinase, *HSP* heat shock protein, *JAK* Janus kinase, *PI3K* phosphatidylinositide 3-kinase, *mTOR* mechanistic target of rapamycin, *STAT* signal transducer and activator of transcription, *TNBC* triple-negative breast cancer

chemotherapy (0 and 10%, respectively) [14]. While meta-analysis of 13 studies ($N = 2826$ TNBC patients) indicated that AR+ TNBC patients had lower grade tumors ($P < 0.001$) and prolonged disease-free survival (hazard ratio [HR], 0.809; 95% confidence interval [CI], 0.659–0.995, $P < 0.05$), these patients also had a higher incidence of lymph node metastases ($P < 0.01$) [6]. Thus, while LAR may be more indolent than the other TNBC molecular subtypes, it may benefit less from chemotherapy (likely because it is less proliferative). Consequently, LAR represents a subtype for which targeted therapy is feasible, given the many drugs either already approved or in development for targeting AR or androgen synthesis in prostate cancer.

Preclinical research on AR in TNBC initially focused on the LAR subtype and high expression of downstream targets of AR signaling [19, 38]. MDA-MB-453 and other LAR cell lines are AR-driven and are sensitive to the early-generation AR antagonist bicalutamide [4, 19]. Additional studies from the Richer Laboratory at the University of Colorado have demonstrated that AR inhibition with enzalutamide in four TNBC cell lines representing three non-LAR TNBC subtypes (BL2, ML, and MSL [mesenchymal stem-like, a TNBC subtype later reclassified [19, 33]]) increased apoptosis and decreased baseline tumor cell proliferation, migration, invasion, and anchorage-independent growth [16]. Furthermore, the predominately nuclear expression of AR in TNBC primary tumors suggests that AR is transcriptionally active [16, 37]. These results suggest that even TNBC subtypes with low AR expression can still critically depend on AR and that it may be the less proliferative AR+ cells that persist and recur. Indeed, in a preclinical model, AR inhibition (enzalutamide) combined with paclitaxel was strikingly more effective at preventing recurrence than paclitaxel alone [22].

While detailed analyses are not yet available, preclinical and clinical results indicate that even TNBC with low AR expression may benefit from AR inhibition. AR pathway activation is likely critical, and in the enzalutamide trial in TNBC, a 35% clinical benefit rate (CBR; the proportion of

patients who experienced a complete response, partial response, or stable disease at 16 weeks of therapy) was observed in patients who had a certain, presumably androgen-driven, gene signature [39, 40] more predictive of response than degree of AR positivity by IHC. This is not terribly surprising, however, since the same is true of ER, where even patients whose tumors express down to 1% ER positivity can receive benefit from anti-estrogen and estrogen deprivation therapies, and ER activity readouts such as PR status, MammaPrint, and Oncotype DX are indicators of the degree of ER dependence. To date, the precise AR-regulated genes essential to TNBC biology remain to be determined and validated as predictive biomarkers of therapeutic benefit.

Androgen Receptor Mutations and Splice Variants in Triple-Negative Breast Cancer

Very few mutations have been found in AR in TNBC compared to castration-resistant prostate cancer (CRPC). Sequencing data in The Cancer Genome Atlas (TCGA) from 93 analyzed TNBCs identified two patients with single missense mutation (described by Barton et al. [15]). The AR mutational status may increase under selective pressure if AR-targeted agents become more commonplace in BC treatment.

AR splice variants that affect AR function are relatively more common in BC than AR mutations [41–44]. One splice variant (Δ3AR) has a deletion of exon 3 and was predicted to lack the second zinc finger within the DNA-binding domain and have reduced or no ability to bind to androgen response elements and activate transcription [41]. In some BC tissues, this AR variant had relatively high expression compared to the full-length protein, indicating a potential role in regulating the growth of these tumors. Another splice variant, AR45, has low expression levels in normal breast tissue [42]. This splice variant lacks exon 1 and is preceded by a novel 7–amino acid long N-terminal extension that inhibits AR function [43]. AR45 and another AR splice variant, AR-V7 (formerly known as AR3), are found in the TNBC cell lines MDA-MB-453 and MDA-MB-231 [44]. Although AR splice variants have been identified in BC cell lines, further studies are needed to characterize AR splice variant expression in TNBC specimens, particularly after the selective pressure of anti-androgen therapy.

The AR-V7 splice variant is of particular interest since it is associated with resistance to anti-androgen therapy in CRPC [45, 46]. This isoform of AR produces a protein product that lacks the C-terminal ligand-binding domain but retains the transcriptionally active N-terminal domain. Although it is unable to bind ligand, AR-V7 is constitutively active in a ligand-independent manner and is capable of promoting activation of target genes. Research on the AR-V7 splice variant in CRPC using circulating tumor cells (CTCs) demonstrates that the presence of AR-V7 is associated with poorer outcomes (prostate-specific antigen [PSA] response, PSA progression-free survival [PFS], clinical or radiographic PFS, and overall survival [OS]) [45–47]. In addition, AR-V7 is associated with better OS with chemotherapy (taxane) than anti-androgen therapy (abiratone, enzalutamide, or apalutamide) [48]. Whether AR-V7 has similar effects in TNBC remains to be seen.

Measurement of Androgen Receptor Protein Expression by Immunohistochemistry

All staining procedures must be standardized and validated clinically and Clinical Laboratory Improvement Amendments (CLIA)-certified in order to be utilized for clinical treatment decisions. IHC methods are well established for clinical use for certain proteins such as ER and HER2 in breast cancer [49], both of which are used as predictive biomarkers when evaluating targeted treatment. The current pathological characterization of AR expression levels in BC is largely based on IHC results using formalin-fixed, paraffin-embedded (FFPE) tissue samples obtained from primary or metastatic tumor biopsies [28] and is not routine or standardized. The detection of AR has improved as more sensitive and specific antibodies to AR have been developed; over time, this has resulted in an increased percentage of TNBC study samples reported as AR+.

In addition to considerations regarding the technical aspects of AR IHC, including the validation and standardization currently underway for BC, tumor biology must also be considered for appropriate interpretation of IHC results. Characterizing AR expression in TNBC accurately can be difficult, in part due to tumor heterogeneity. While tumor heterogeneity is not unique to TNBC, the extent of heterogeneity in TNBC between molecular subtypes and even intratumoral genomic heterogeneity makes defining common features of this type of BC particularly challenging [37]. Another important consideration is whether the patient received prior treatment, and if so, what type of treatment. Certain therapies, such as aromatase inhibitors, increase circulating androgen levels [50–55]. AR protein is stabilized and translocated to the nucleus in breast cancer upon binding to androgens [56, 57], and this effect is abrogated by the new-generation anti-androgen enzalutamide (Fig. 2). The effect was observed in ER+ MCF7 cells and the LAR BC cell line MDA-MB-453 grown as xenografts in mice treated with estradiol versus DHT [4]. Likewise, in the non-LAR but AR+ SUM159PT TNBC cell line grown in cycling mice, AR was nuclear and decreased with anti-androgen [16]. In two patient-derived xenografts (PDX) originally low for nuclear AR by IHC, AR increased in mice given DHT versus cellulose control (Fig. 2),

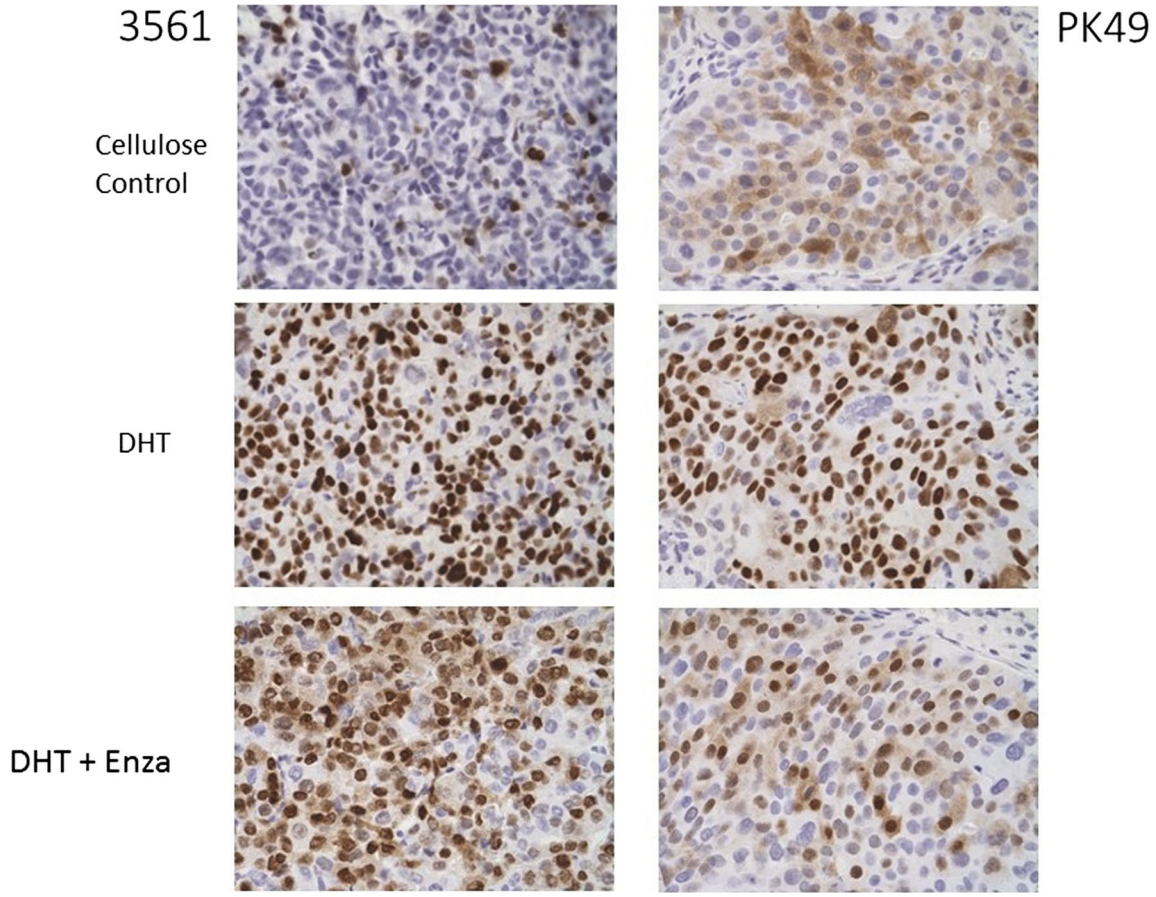

Fig. 2 Two TNBC patient-derived xenografts grown in mice had low AR, but nuclear AR increased upon exposure of mice to DHT and this effect was reduced by subsequent treatment with the anti-androgen enzalutamide. Mice were implanted with silastic tubing containing either cellulose only (10 mg) or a mixture of cellulose and DHT (2 and 8 mg, respectively) at the time of placement of TNBC PDX tumor pieces into the mammary glands. When tumors reached an average of 55 mm^3 in size, mice were either continued on DHT alone or given DHT + Enza in chow fed ad libitum for a target dose of 50 mg/kg/day for 3.5 weeks. FFPE tumors (PDX 3561 and PDX PK49) were stained for AR (SP107, CellMarque, ×40 magnification). *AR* androgen receptor, *DHT* dihydrotestosterone, *Enza* enzalutamide, *PDX* patient-derived xenografts, *TNBC* triple-negative breast cancer, *FFPE* formalin-fixed paraffin-embedded

indicating that even TNBCs that have low AR have the capacity to respond to androgen agonists and antagonists. Currently, there are insufficient data to ascertain how well AR IHC results will correlate with measures of AR dependency and clinical outcome, as well as the minimum percentage of cells positive for nuclear AR that should be considered as AR+, and predictors of response to anti-androgen therapy.

Finally, regarding AR status, it is also important to consider the influence of AR splice variants on staining results. In an overview of AR splice variants found in prostate cancer, the authors noted that several of these variants lack the C-terminal ligand-binding domain [58]. AR signaling is constitutively active in such variants, potentially contributing to resistance to androgen deprivation therapy (ADT) [58–61]. Additionally, AR has many phosphorylation sites, although the contribution of these sites to transcriptional activity is not yet as clear as it is for ER, where there clearly are sites phosphorylated upon addition of ligand that affect receptor activity and turnover.

New Methods for Determining Androgen Receptor Status

Alternative approaches to clinical characterization of AR+ BC are currently in the early stages of development. Given the long-term understanding of the role of androgens in prostate cancer pathogenesis, several novel approaches are being investigated, including blood-based methods using CTCs, circulating tumor DNA (ctDNA), and exosomes [62]. These blood-based approaches allow for more frequent and less invasive assessments, which are helpful for diseases like CRPC and TNBC, where AR status can change over the course of disease progression [45, 46]. The less invasive approaches of liquid biopsies are especially useful for difficult to biopsy lesions, as described below in a case study.

In prostate cancer, CTCs can be used to identify mutations in AR, AR expression and function, and response to therapy [63–66]. In one study of progressive metastatic CRPC, evaluation of CTCs revealed that AR expression and nuclear

localization varied both within and between patients, suggesting that a molecularly diverse, AR-centric pathobiology underlies castration resistance [66]. In another study of CRPC, CTC-based assays were used to track AR expression in real time in patients treated with enzalutamide and abiraterone, another inhibitor of androgen synthesis [67]. For BC, a presentation at the 2016 annual meeting of the American Association of Cancer Research reported characterization of AR expression and heterogeneity in CTCs of patients with metastatic BC [68].

Similarly, ctDNA has been used to identify AR mutations in CRPC. Two studies have examined a missense mutation in the ligand-binding domain of AR that conferred resistance to the second-generation anti-androgens enzalutamide and apalutamide (ARN-509) [69, 70]. There is growing use of ctDNA techniques in BC in place of traditional tumor biopsies [71], and similar to CRPC, it could be a valuable tool to determine AR status.

In addition, research on prostate cancer suggests that exosomes may serve as potential biomarkers of AR status as well as predictors of therapeutic response. Exosomes act as mediators of cell-to-cell communication in the local tumor microenvironment and play a role in cancer progression and metastasis [72, 73]. One study demonstrated that AR is present in prostate cancer-related exosomes [74]. Furthermore, patients with aggressive prostate cancer exhibited higher levels of prostate cancer-related exosomes in the blood than prostate cancer patients without metastases or healthy volunteers [74]. In another study, plasma-derived exosomal RNA was used to detect the presence of an AR splice variant and predict resistance to hormonal therapy in metastatic CRPC patients [75]. Future experiments will determine if similar results are observed in BC patients.

As an example, a case study in which a blood-based method was used to determine a breast cancer patient's AR status is presented below.

Case Study of Liquid Biopsy Analysis to Determine Current AR Status in a TNBC Patient with an Inaccessible Lesion

AR expression in TNBCs is likely to be indicative of a tumor that may respond to AR-targeted therapy. However, for a variety of reasons, it is likely that archival FFPE tumor samples may not be indicative of the current AR expression level, and patients with metastatic disease may not have accessible tumor for reassessment. It is crucial to identify biomarkers predictive of response to anti-androgens in TNBC. This patient vignette illustrates how peripheral blood, which is easily accessible, can be used to quantify AR utilizing a digital assay that counts copies of AR mRNA. Not only can this assay provide a potential predictive biomarker for AR-targeted therapy, it can also dynamically follow the level of AR in a patient during the course of their therapy.

The subject was a 63-year-old female initially diagnosed with TNBC in June 2011 who underwent a left breast mastectomy. She was disease-free until 2014; upon disease recurrence, she was treated with capecitabine from May to December 2014, followed by nab-paclitaxel plus carboplatin combination therapy from December 2014 to May 2015 and then nab-paclitaxel as a single agent from June 2015 to January 2016. The subject had progressive visceral (lung) disease and was enrolled into the phase 1 portion of CLARITY-01, the phase 1/2 study of seviteronel, in February 2016. She was considered AR− at study entry based on IHC of an archival FFPE sample from a metastatic lymph node biopsy obtained in 2014. Eastern Cooperative Oncology Group (ECOG) performance status at screening was 1.

The subject began seviteronel dosing at 450 mg once daily in 28-day continuous dosing cycles and responded to treatment with radiographic stable disease for almost six 28-day cycles. To better understand the AR status of her current disease state, the subject was scheduled to have a lung metastasis biopsy, but the procedure was considered unfeasible due to the tumor location. Instead, peripheral blood was collected in a PAXgene tube for RNA stabilization, and a sensitive, digital assay for AR mRNA using the droplet digital PCR (ddPCR) platform demonstrated a high level of AR positivity. This provided a real-time, minimally invasive assessment of the cancer cell AR status in a patient who met the criteria for clinical activity (complete response, partial response, or stable disease at 16 weeks of therapy) in the clinical study.

To establish the assay, AR full-length and AR-V7 splice-variant mRNA copy numbers were determined by ddPCR analysis of peripheral blood drawn into PAXgene blood RNA tubes. Cutoffs were established by comparison with healthy controls and patients with localized prostate cancer, stage D0 prostate cancer, and metastatic prostate cancer. These studies allowed development of an AR-V7 cutoff that correlated with metastatic disease. Previous studies have utilized mononuclear cells isolated from peripheral blood and ddPCR analysis [76] or whole blood collected in PAXgene blood RNA tubes and quantitative PCR analysis [77].

Androgen Receptor and Epithelial-to-Mesenchymal Transition

Epithelial-to-mesenchymal transition (EMT) is a process through which normal or carcinoma cells can lose cell-cell junctions/polarity and develop a more migratory, stem cell-like phenotype [78]. While this process is critical during embryonic development, it has also been implicated at certain steps in the metastatic cascade [79]. In BC, AR contributes to EMT and metastasis in several ways. Loss of E-cadherin, an epithelial

marker, is a common EMT event that helps promote metastasis [80]. In ER+ BC, AR activation causes a decrease in E-cadherin and an increase in stem cell-like properties and EMT-related gene expression as well as an increase in metastasis [81, 82]. These results are supported by recent evidence linking activated AR to acquisition of a stem-like phenotype in TNBC cells and increased MDA-MB-231 xenograft growth [83]. In TNBC, AR promotes survival in anchorage-independent conditions and maintains a CSC-like tumor-initiating population [22]. Correspondingly, in TNBC PDX models, AR mRNA was among the transcripts upregulated in CTCs and micrometastases as compared to primary tumors [18]. Furthermore, in a mouse mammary tumor virus-polyoma middle tumor antigen (MMTV-PyMT) model, ER and PR proteins are absent but AR protein is abundant in lung metastases and AR inhibition significantly decreased cancer cell invasion and anchorage-independent growth in vitro [84]. Together, these data suggest that AR may facilitate BC metastasis by protecting against apoptosis in an anchorage-independent tumor cell population with EMT or stem cell-like properties.

The association between AR and EMT has important clinical implications. While a large percentage of TNBC patients responds favorably to chemotherapy, many will relapse with chemoresistant disease since chemotherapy often fails to target the slower growing population of cells (that are either CSC-like or more epithelial, as in the case of LAR TNBC). If AR promotes EMT and stemness, then the combination of AR inhibitors and chemotherapy may be a rational and impactful drug combination for patients. Preclinical data in a TNBC xenograft model support this hypothesis, demonstrating that the combination of paclitaxel and enzalutamide given simultaneously significantly decreased tumor growth and recurrence when compared to paclitaxel alone [22].

The Influence of Androgen Receptor on Immune Oncology

AR expression in cells within the tumor microenvironment could have significant effects on tumor growth and progression. AR is expressed in a number of immune cells, both innate and adaptive, and knockout of AR can have profound effects on immune cell maturation and function [85–88]. In particular, AR activation alters T cell immunity by suppressing T cell (CD4 and CD8) proliferation and inhibiting CD4 T-helper differentiation [89, 90]. Given that T cells play a prominent role in anti-tumor immunity and that T cell infiltration is a predictive marker in TNBC [91–93], the systemic use of anti-androgens (or androgen agonists) could have significant effects on anti-tumor immune activity. It was previously reported that androgen deprivation in prostate cancer patients leads to an increase in T cell infiltration into the prostate [94]. More recent studies investigated the effects of anti-androgens on CD8 T cell anti-tumor activity in both prostate cancer and BC [59, 95–97]. To date, however, it remains unclear how long-term AR targeting therapies will affect the immune system and whether they will boost or be detrimental to anti-tumor immunity. It would be particularly beneficial if endocrine therapy proved useful in combination with targeted immunotherapies such as PD-L1 (programmed death ligand 1) interfering antibodies [98, 99].

Targeted Agent Activity Alone and in Combination for Treatment of AR+ Triple-Negative Breast Cancer

With AR possibly playing a central role in AR+ TNBC tumorigenesis, AR-targeted agents, such as androgen biosynthesis inhibitors (eg, cytochrome P450 C17a [CYP17] inhibitors) and inhibitors of AR activation (eg, AR antagonists), are being examined in clinical trials [100] (Table 1). To date, data are available from studies of bicalutamide, enzalutamide, seviteronel, and abiraterone acetate, and a study of orteronel (TAK-700) is in progress [101].

The first trial of an anti-androgen in BC was a phase 2 trial of bicalutamide in AR+/ER− metastatic BC. Bicalutamide, long used to treat prostate cancer, is a competitive antagonist that permits AR nuclear translocation and binding to DNA but in an inactive form [102]. In a phase 2 trial, 5 of 26 patients with AR+/ER−/PR− BC treated with bicalutamide had stable disease for at least 6 months, resulting in a 24-week CBR of 19% [103]. AR expression in the 5 patients was varied: 10−20% (1 patient), > 50% (1 patient), > 80% (2 patients), and > 90% (1 patient). Median PFS was 12 weeks (95% CI, 11–22 weeks), and the most common drug-related adverse events (AEs) were fatigue, hot flashes, limb edema, and aspartate aminotransferase or alkaline aminotransferase elevations. This was the first clinical trial to establish the activity of anti-AR therapy in advanced BC and the potential of targeting AR in AR-dependent, ER-independent BC [103]. However, while disease stabilization in 5 patients with AR+/ER– metastatic BC is indicative of AR inhibition, it is also possible that these 5 patients had more indolent disease since LAR TNBCs are less proliferative than other subtypes of TNBC.

The next trial of an AR inhibitor in TNBC was with enzalutamide, a newer-generation AR competitive inhibitor approved by the FDA to treat men with metastatic CRPC [57]. Enzalutamide, like bicalutamide, directly binds to AR and is a competitive antagonist but has a > fivefold higher binding affinity than bicalutamide and, in contrast to bicalutamide, impairs AR nuclear translocation, inhibits AR-DNA binding and gene regulation, and consequently has no partial agonist activity [56, 104, 105]. In a phase 2 study of enzalutamide in advanced AR+ TNBC, 26 (35%) of 75 evaluable patients demonstrated a 16-week CBR, and 22 (29%) demonstrated a 24-week CBR [39]. Of the 26 patients

Table 1 Clinical trials of AR-targeted therapies in TNBC

NCT no.	Phase	Patient population	Treatment	Treatment type	Start date	Status note
NCT00468715	II	Metastatic AR+ TNBC	Bicalutamide	AR antagonist	Mar 2007	
NCT02348281	II	Advanced AR+ TNBC; PM	Bicalutamide	AR antagonist	Jan 2015	Terminated
NCT03055312	III	Metastatic AR+ TNBC	Bicalutamide	AR antagonist	Dec 2016	
NCT02605486	I/II	Metastatic AR+ BC	Bicalutamide + palbociclib	AR antagonist + CDK4/6 inhibitor	Nov 2015	
NCT03090165	I/II	Advanced AR+ TNBC	Bicalutamide + ribociclib	AR antagonist + CDK4/6 inhibitor	Mar 2017	
NCT01889238	II	Advanced AR+ TNBC	Enzalutamide	AR antagonist	June 2013	
NCT02750358	II	Early stage AR+ TNBC; adjuvant	Enzalutamide	AR antagonist	May 2016	
NCT02676986	II	AR+ BC; neoadjuvant	Enzalutamide (± exemestane)	AR antagonist (±AI)	Aug 2015	
NCT02689427	IIB	AR+ TNBC; neoadjuvant	Enzalutamide ± paclitaxel	AR antagonist ± chemotherapy	Sep 2016	
NCT02929576	III	Advanced AR+ TNBC	Enzalutamide ± paclitaxel	AR antagonist ± chemotherapy	Sep 2016	Withdrawn
NCT02457910	I/II	Advanced AR+ TNBC; PM	Enzalutamide ± taselisib	AR antagonist ± PI3K inhibitor	June 2015	
NCT03207529	I	Metastatic AR+ BC; PTEN+	Enzalutamide ± alpelisib	AR antagonist ± PI3K inhibitor	Dec 2017	
NCT01842321	II	Advanced AR+ TNBC	Abiraterone + prednisone	CYP17 inhibitor + corticosteroid	July 2013	
NCT01990209	II	Metastatic AR+ BC	Orteronel	CYP17 inhibitor	Mar 2014	
NCT02580448	I/II	Advanced AR+ BC; ER+/HER2− and TNBC	Seviteronel	CYP17 inhibitor/AR antagonist	Aug 2015	
NCT02067741	II	Metastatic AR+ BC; ER+/HER2− and TNBC	CR1447 (4-OH-testosterone)	AR agonist	May 2016	
NCT02000375	II	Metastatic AR+ BC; ER+ and TNBC, PM	DHEA	AR agonist	Mar 2013	Terminated
NCT02368691	II	Advanced AR+ TNBC	GTx-024	Selective AR modulator	June 2015	Terminated
NCT02971761	II	Advanced AR+ TNBC	GTx-024 + pembrolizumab	Selective AR modulator + anti-PD-1	June 2017	

AI aromatase inhibitor, *AR* androgen receptor, *BC* breast cancer, *CDK* cyclin-dependent kinase, *CYP17* cytochrome P450 17, *DHEA* dehydroepiandrosterone, *ER* estrogen receptor, *HER2* human epidermal growth factor receptor 2, *NCT* national clinical trial, *PD-1* programmed cell death protein 1, *PI3K* phosphoinositide 3-kinase, *PM* postmenopausal, *PTEN* phosphatase and tensin homolog, *TNBC* triple-negative breast cancer

who had CBR at 16 weeks, 2 had a complete response and 5 patients had a partial response. Median PFS was 14 weeks (95% CI, 8–19 weeks). The most common therapy-related AEs were fatigue, nausea, decreased appetite, diarrhea, and hot flush.

The non-steroidal CYP17 inhibitor orteronel (TAK-700) was initially being developed for the treatment of CRPC but failed in phase 3. It is currently being investigated in women with AR+ TNBC [101].

A phase 2 trial investigated the efficacy of abiraterone acetate—an irreversible and potent inhibitor of CYP17—with prednisone in women with metastatic or inoperable locally advanced AR+ TNBC [106]. Of 30 patients who were eligible and evaluable for the primary endpoint, 6 (20%) had a CBR at 6 months. Of these 6 patients, 1 had a complete response and 5 had stable disease. However, this proportion of patients achieving clinical benefit was insufficient to meet predefined criteria to reject the null hypothesis. The most common drug-related AEs were fatigue, hypertension, hypokalemia, and nausea, with the majority being grade 1 or 2.

In addition, the clinical benefit of seviteronel, a non-steroidal selective CYP17 17,20 lyase and AR inhibitor that blocks both testosterone and estradiol production and inhibits AR activation, was recently reported from an ongoing phase 1/2 study that includes a separate cohort of women with unresectable locally advanced or metastatic AR+ TNBC in addition to ER+/HER2− BC [107]. The 16-week CBR for AR+ TNBC was 2 of 6 patients (33%), allowing full stage 2 accrual. Declines in CTCs were observed in 7 of 10 evaluable patients (AR+ TNBC and ER+ BC patients), including all patients who met clinical benefit criteria across both cohorts. The most common AEs were fatigue, dizziness, nausea, and decreased appetite, all grade 1 or 2.

The first-generation AR antagonist bicalutamide and the next-generation AR antagonist enzalutamide have both demonstrated clinical activity in patients with AR+ TNBC, as discussed above. Seviteronel, with a dual mechanism of action of selective CYP17 lyase inhibition and AR antagonism, also has demonstrated initial clinical activity and full phase 2 clinical development is ongoing in women with AR+ TNBC in addition to other types of male and female BC [108]. Thus, a strategy of combined targeting of androgen biosynthesis and AR inhibition appears promising for the treatment of AR+ TNBC.

Other combination strategies utilizing AR-targeted agents with non-endocrine agents are also under clinical investigation in AR+ TNBC. For example, AR antagonism in AR+ TNBC is being explored in combination with a cell cycle cyclin-dependent kinase CDK4 and CDK6 inhibitor (bicalutamide + palbociclib) [109] and with a phosphoinositide 3-kinase (PI3K) inhibitor (enzalutamide + taselisib) [110]. The combination of AR antagonists and taxanes is also being investigated [111] since taxanes have been shown to inhibit the translocation of the AR from the cytoplasm to the nucleus in prostate cancer [112] and were effective together in preventing recurrent disease in a preclinical model of TNBC [22]. Further work is needed to develop rational combinations that utilize AR-targeted agents in AR+ TNBC.

Conclusion

Most types of BC, including TNBC, can be driven in part by activated, ligand-bound AR. While IHC using FFPE samples is the traditional means of measuring AR expression in tumors, new blood-based approaches may provide improved real-time AR assessment. Several novel drugs are currently in development that target AR and/or androgen production that may provide additional options for patients with AR+ TNBC for whom chemotherapy is the only current treatment option.

As preclinical research strives to better model the clinical situation, varied approaches are being taken, such as utilization of patient-derived xenografts and mouse mammary carcinoma models with intact immune systems derived from genetically engineered transgenic models, spontaneous arising tumors, or chemically induced tumor models to examine the effects of AR inhibition on both the anti-tumor immune response and the immune system in general. There remains much to be learned regarding how to leverage the impact of endocrine therapy (even in TNBC) on host anti-tumor immunity and develop optimal combination regimens with other therapies for TNBC. There is also evidence that anti-androgens may have off-target effects [97], and research into these alternative mechanisms of action is ongoing. In conclusion, modeling various clinically relevant physiological states such as pre- or postmenopause, postpartum pregnancy-associated BC, and obesity in immune-intact animals is an important direction for future research and will address contemporary questions.

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Compliance with Ethical Standards

Conflict of Interest JRE and ESBB declare that they are employed by and have stock ownership in Innocrin Pharmaceuticals, Inc. All other authors declare that they have no potential conflict of interest.

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