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Targeting the androgen receptor in triple negative breast cancer

Ayca Gucalp^{1,2} and Tiffany A Traina^{1,2}

¹Memorial Sloan Kettering Cancer Center, New York, NY

²Weill Cornell Medical College, New York, NY

Keywords

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Introduction

Triple negative breast cancer (TNBC), a relatively new term first published in 2005[1], is used to describe a subset of breast cancers characterized by the absence of expression of the estrogen receptor (ER), progesterone receptor (PgR), and HER2 protein. TNBC represents approximately 15–20 % of all newly diagnosed breast cancers but accounts for a disproportionate number of breast cancer-related deaths each year making up 5% of all cancer deaths annually.[2, 3] Due to the lack of receptors, patients with TNBC do not benefit from generally well-tolerated and effective therapies targeting the ER and HER2 and often experience a more aggressive clinical course with increased risk of disease progression and poorer overall survival.[4] A subset of TNBC tumors express the AR and may benefit from treatments that inhibit the AR-signaling pathway. This review summarizes the underlying biology of AR signaling in breast cancer development and the available clinical trial data for the use of antiandrogen therapy in the treatment of AR+ TNBC.

Structure and function of the androgen

AR is a member of the steroid-hormone family of receptors which also includes the estrogen, progesterone, glucocorticoid, and mineralocorticoid receptors. Located on chromosome Xq11–12, physiologically AR-regulated signaling is responsible for male sexual differentiation and reproductive development. In adult males, androgens maintain libido, spermatogenesis, muscle mass and strength, bone mineral density, and erythropoiesis. The gene for AR encodes a 110kDa polypeptide with four distinct functional regions: (i) an N-terminal region involved in transcriptional activation (ligand-independent activation function 1 (AF1) domain), (ii) a DNA-binding domain composed of two zinc fingers that

Corresponding Author: Tiffany A. Traina, M.D., Address: Breast Medicine Service, Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, Evelyn H. Lauder Breast Center, 300 East 66th Street, New York, NY 10065, trainat@mskcc.org.

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interacts with androgen response elements, (iii) a ligand-binding domain (LBD) to which androgens and antiandrogens bind which includes an androgen-dependent activation function 2 domain at the C-terminus), and (iv) a hinge region that connects the DNA-binding domain and LBD, and controls nuclear localization. Unbound AR resides primarily in the cytoplasm, bound to chaperone proteins such as heat shock proteins that stabilize the receptor in a conformation that promotes ligand binding. In the presence of androgens (testosterone and dihydrotestosterone (DHT)), the AR undergoes a series of conformational changes, dissociates from the heat shock proteins, and forms a homodimer that translocates to the nucleus. In the nucleus the AR-complex binds to androgen response elements and recruits co-regulatory activators leading to the activation of target gene transcription[5–7].

Published data supports that the androgen-signaling pathway has a role in breast cancer development. Notably, both growth stimulatory and inhibitory effects of androgens have been described in breast cancer cells lines [8, 9]. Reconciling these seemingly paradoxical effects is complex. However, emerging data suggests that the function of the AR in breast cancer pathogenesis may be in part dependent on the underlying molecular phenotype of the tumor, the relative coexpression of other hormone receptors, and the hormonal environment. Historically androgens, such as fluoxymesterone, testolactone, and calusterone have been used for the treatment of advanced breast cancer resulting in clinical responses ranging from 18–39% [10–13]. However, the undesirable virilizing side effects of these agents have limited their routine use in the treatment of breast cancer particularly in the advent of newer, less toxic endocrine agents. In contrast several clinical trials have now demonstrated the activity of anti-androgen therapy for the treatment of AR+ TNBC.[14–16]

The mechanism by which androgen signaling influences hormonal sensitivity and breast carcinogenesis remains incompletely characterized and an area of active research. The following sections will focus on the recent progress made in further understanding this subgroup of TNBC characterized by AR expression, the underlying mechanisms by which AR driven signaling may influence the development of this subtype of breast cancer, and the available clinical data regarding the use of anti-androgen therapy for the treatment AR+ TNBC.

Androgen receptor in the pathogenesis of triple negative breast cancer

Farmer and colleagues described the existence of a subset of ER/PgR (–) tumors that demonstrated increased androgen signaling, a molecular apocrine gene expression profile, and increased co-expression of HER2, differentiating these tumors from the classic basal phenotype that had previously been described.[17–20] Independently, researchers at Memorial Sloan Kettering Cancer Center identified a similar subset of hormone receptor (HR)-negative, AR+ breast cancer. Analyzing the gene expression profiles of 99 archived primary breast tumors, Doane et al showed that this subgroup, named ER(–) class A, was distinguished by AR expression, a hormonally driven transcription program and androgen-dependent and estrogen-independent growth. Gene expression signatures were then used to develop a predictive model to identify molecularly similar breast cancers in an independent human breast cancer data set and among breast cancer cell lines. In order to evaluate the functional role of the AR in this subset of tumors, in vitro studies were carried out on the

MDA-MB-453 breast cancer cell line which has a molecular phenotype similar to that of the ER(-) class A subtype. Exposure to estrogen failed to produce measurable cell growth in the MDA-MB-453 cell line and subsequent exposure to antiestrogens did not have any effect, further reducing the likelihood that growth in this tumor subgroup was estrogen-dependent. In comparison, incubation with the synthetic androgen, R-1881, resulted in cell proliferation which was then abrogated by the AR-inhibitor flutamide, suggesting an AR-dependent mechanism of cell growth in this cell line.[21]

Several other groups have subsequently described this unique subset of HR(-) tumors.[22–27] Evaluating over 500 samples from patients with TNBC, Lehmann and colleagues described significant heterogeneity within TNBC. One of the 6 subgroups they described, termed the luminal androgen receptor (LAR) subtype was characterized by high expression of the AR mRNA and protein, AR downstream effectors/coactivators, and luminal cytokeratins/markers, and lacking basal cytokeratin expression. The MDA-MB-453 cell line along with three additional cell lines (SUM185PE, CAL-148, and MFM-223), identified through gene expression profiling to have a molecular phenotype similar to that of LAR tumors, were used to further assess the functional role of the AR in this ER/PgR(-) subset. As anticipated LAR cell lines were significantly more sensitive to bicalutamide exposure than other TNBC cell lines ($IC_{50}=227\text{ }\mu\text{M}$ vs $IC_{50}>600\text{ }\mu\text{M}$; $P=0.007$). Xenograft murine models representative of LAR breast cancer were significantly growth inhibited when treated with bicalutamide relative to treatment with vehicle control or other agents. Moreover transfection of LAR cell lines with AR siRNA allowed investigators to show decrease in colony formation in the cell lines with knockdown AR expression in comparison to controls (MFM-223=55%, $P=0.031$; MDA-MB-453=51%, $P=0.004$; SUM185PE=42.3%; $P=0.002$). This preclinical work further substantiated the hypothesis that tumor growth in this subtype is dependent on androgen signaling and that AR expression may be necessary for cell survival and proliferation. Subsequently others have studied the effects of next generation AR-inhibitors such as enzalutamide in LAR cell lines and mouse models with similar results.[28] Collectively these data set the stage for investigators to evaluate the utility of targeting the AR-signaling pathway in the treatment of this subgroup of TNBC.

Androgen receptor as a therapeutic target in triple negative breast cancer

The current standard of care in the treatment of TNBC offers limited therapeutic options aside from traditional cytotoxic chemotherapy agents. Treatments that target the androgen-signaling pathway have the potential to allow a greater number of patients with advanced or metastatic TNBC the opportunity to be treated with effective, less toxic endocrine agents.

Monotherapy

Bicalutamide—Building upon the preclinical work of Doane and colleagues, investigators at MSK led a multicenter, single arm, phase II trial through the Translational Breast Cancer Research Consortium (TBCRC) to test the hypothesis that AR-inhibition with bicalutamide (150 mg daily) had antitumor activity in patients with advanced or metastatic AR+ ER/ PgR(-) breast cancer (NCT00468715/TBCRC011). Bicalutamide is an orally available nonsteroidal competitive AR inhibitor that is approved by the US Food and Drug

Administration (FDA) for the treatment of advanced prostate cancer in combination with a luteinizing hormone-releasing hormone analogue. The primary endpoint was clinical benefit rate (CBR) defined as complete response (CR), partial response (PR), or stable disease (SD) > 6 months. More than 450 patients were screened for AR status; ~12% of patients had >10% nuclear expression of AR by immunohistochemical testing of either their primary tumor or a metastatic site. Daily treatment with bicalutamide was well-tolerated and resulted in a CBR of 19% with a median PFS of 12 weeks in patients in this population. A subset of patients demonstrated prolonged stable disease, measured in years and one patient with unresectable, locally advanced disease following neoadjuvant anthracycline- and taxane-based therapy was able to achieve definitive local therapy following treatment with bicalutamide.[14] These data are a signal of activity for androgen blockade in AR+ ER/PgR – breast cancer and serve as a proof of concept.

The development of novel, next-generation compounds which inhibit AR activity for the treatment of metastatic castrate resistant prostate cancer (mCRPC), such as enzalutamide and abiraterone acetate, led to the hypothesis that they may offer superior clinical outcomes for AR-dependent breast cancers.

Enzalutamide—Enzalutamide is a pure androgen receptor signaling inhibitor. In addition it inhibits androgen receptor nuclear translocation, DNA binding, and coactivator mobilization. Unlike other antiandrogen agents, it has no known agonistic properties. The FDA approved enzalutamide for the treatment of patients with mCRPC who have previously received docetaxel based on a randomized, placebo-controlled, multicenter trial which demonstrated an improvement in overall survival in this population as compared to placebo. Additional phase II studies have shown enzalutamide to be superior to bicalutamide in terms of progression-free survival (PFS) in a similar patient population [29, 30]. In a phase I dose-escalation study in patients with metastatic breast cancer (MBC), enzalutamide was found to be well tolerated at 160 mg daily with common treatment-related adverse events including nausea, vomiting and fatigue [31, 32].

For patients with AR+ metastatic TNBC, a phase II study of single-agent enzalutamide 160 mg daily has completed accrual (NCT01889238). Similar to TBCRC011 the primary endpoint of this study CBR defined here as CR+PR+SD at 16 weeks in the evaluable patient population. Individuals with AR staining >10% and having one post-baseline assessment available were considered evaluable. The trial met its primary endpoint with a CBR at 16 of 35% and a median PFS of 14.7 weeks. In addition two CRs and five PRs were reported in response to enzalutamide therapy. The most common adverse events were fatigue, nausea and decreased appetite. The only common (5%) grade 3 or greater adverse event related to study agent was fatigue. There were no serious adverse events reported in this population.

Recognizing that AR by IHC may not be the ideal predictor of benefit to anti-androgen therapy one of the key exploratory endpoints of the study was the development of a biomarker to predict response to enzalutamide in this population. Forty-seven percent of patients in the intention to treat (ITT) population (n=118) were found to be positive for the biomarker (Dx). Stratifying by Dx status in the ITT population, patients with Dx+ TNBC experienced more favorable outcomes in all response measures including CBR16 (39% vs

11%), CBR24 (36% vs 6%) and median PFS (16.1 weeks vs 8.1 weeks) when compared to their biomarker negative counterparts. This trial further reinforced the potential utility of anti-androgen therapy for the treatment of a subset of patients with TNBC, providing the first objective RECIST-confirmed responses to an AR-antagonist and advancing the development of an assay to help better select patients with TNBC who may benefit from therapy with enzalutamide.

The ENDEAR study is a phase III trial evaluating the efficacy enzalutamide in patients with Dx+ TNBC. Unlike prior trials in this patient population eligibility is not based on AR expression by IHC but instead on the novel gene expression profile developed as a companion diagnostic during the phase II trial described above. Patients with DX+ TNBC will be randomized in a 1:1:1 to receive enzalutamide monotherapy or paclitaxel+placebo or dual therapy with paclitaxel+enzalutamide. The primary efficacy endpoint is PFS. The trial is expected to begin enrollment in late 2016.

Based on the encouraging data available in the metastatic setting, a phase II study is currently enrolling patients to evaluate the feasibility of enzalutamide therapy in patients with early stage, AR+ TNBC (NCT02750358; Table 1). The use of extended adjuvant oral endocrine therapy is a standard of care for the treatment of patients with ER+ early stage breast cancer based on improvements in disease free and overall survival. However patients with AR+ TNBC do not benefit from anti-estrogen therapy and available cytotoxic therapies remain unfortunately inadequate. Ultimately the aim will be to assess whether patients with AR+ TNBC may similarly benefit from an adjuvant antiandrogen approach as these tumors appear to be driven by AR-signaling rather than ER-signaling. However the primary endpoint for this phase II trial is feasibility defined by the discontinuation rate of enzalutamide for 1 year due to toxicity, withdrawal of consent, or other causes related to tolerability.

Abiraterone Acetate—Abiraterone acetate is a potent, orally available, selective inhibitor of both 17 α -hydroxylase and c17, 20-lyase, which targets adrenal and tumor intracrine androgen biosynthesis. Abiraterone acetate in combination with prednisone is FDA approved for the treatment of advanced prostate cancer in men who are both chemotherapy-naïve disease and following progression on docetaxel. Based on the mechanism of action of abiraterone acetate as a CYP17 inhibitor, there is a rationale for studying its potential benefit in AR+ TNBC, as reduced levels of androgens are expected from upstream inhibition of the steroid synthesis pathway. To assess the safety and efficacy of abiraterone acetate in combination with prednisone a phase II multicenter trial of was conducted through the French cooperative group UNICANCER in women with metastatic or inoperable locally advanced AR+ TNBC. One hundred thirty-eight patients with adequate tissue were AR tested of which 53 (38.4%) were AR-positive and triple-negative. From July 2013-December 2014, Bonnefoi and colleagues treated 34 women with AR+ TNBC. Ultimately 30 patients were deemed eligible and evaluable for the primary endpoint of CBR at 6 months. Patients were considered evaluable if they completed at least one cycle of therapy and underwent having at least one disease assessment at eight weeks after initiating study treatment. CBR at 6 months was 20.0% (95% CI: 7.7–38.6%), including 1 CR and 5 SD 6 months. Median PFS was 2.8 months (95% CI: 1.7–5.4%). Similar to TBCRC011, the subgroup of patients

who achieved clinical benefit were generally older, without visceral involvement, and had received less lines of therapy in the metastatic setting. As expected, the most common drug-related adverse events included fatigue, hypertension, hypokalemia and nausea. While grade 3 adrenal insufficiency, hypokalemia, and hypertension were reported in this population, there were no grade 4 or 5 adverse events. Although the study did not meet its primary endpoint, 2 objective responses were documented in this population suggesting that coadministration of abiraterone acetate and prednisone may be beneficial for a select population of patients with molecular apocrine-like tumors.

Ongoing clinical trials targeting the AR-signaling pathway

The development of agents that target the AR-signaling pathway for the treatment of TNBC is an area active investigation. A number of other novel antiandrogens are currently undergoing evaluation in clinical trials either as monotherapy or in combination with other targeted agents (Table 1).

Seviteronel—Another androgen-directed therapy under investigation for the treatment of AR+ TNBC is seviteronel. Seviteronel is an oral, CYP17-L inhibitor and AR antagonist with activity in castration resistant prostate cancer (CRPC) at a dose of 750 mg nightly. Preclinical data demonstrates that seviteronel potently inhibits the growth of ER(-)/AR(+) MDA-MB-453 breast cancer in a dose-dependent manner. The phase I dose-escalation study in patients with advanced or metastatic breast cancer has been completed. Preliminary pharmacokinetic data presented at the annual American Society of Clinical Oncology (ASCO) meeting in 2016 suggests seviteronel bioavailability may be gender-specific and related to body mass/surface area in women. Overall seviteronel was well-tolerated and 450 mg was selected as the recommended phase II dose in women[33]. The phase II in this population is currently enrolling patients (NCT02580448).

Dual inhibition of AR and other signaling pathways

Investigation of the AR pathway in the development of breast cancer has led to the identification of additional signal transduction pathways with the potential to regulate AR downstream activity. This network of signaling pathways, (e.g. PI3K/AKT/mTOR and cell-cycle regulators) are hypothesized to work in concert with AR to regulate cell proliferation and tumor growth. Several clinical trials have been developed to test whether dual-inhibition of these signaling pathways will improve outcomes for patients with androgen-drive TNBC.

Dual CDK4/6 and AR inhibition—The rationale for combining palbociclib with AR blockade stems from published data in patients with advanced ER+ breast cancer. Palbociclib is an orally administered potent and highly selective reversible inhibitor of CDK4/6 which been shown to significantly improve median PFS in two large phase III studies in combination with either letrozole (PALOMA-2)[34] or fulvestrant (PALOMA-3) [35] when compared to monotherapy for the treatment of postmenopausal pts with ER+ MBC. Palbociclib prevents cellular DNA synthesis by prohibiting progression of the cell cycle from G1 into the S phase, as demonstrated in laboratory models [36]. Preclinical data indicate that the compound may be expected to have direct cytotoxic activity as well as potential for growth arrest. Arrest in the G1 phase is known to cause cell death in some

tumor types, as demonstrated by this and other G1 arresting agents (e.g., tamoxifen). Moreover, CDK inhibitors like palbociclib have the potential to enhance the effects of conventional therapies. For example, palbociclib is synergistic with tamoxifen in ER-positive cell lines with a mean combination index <1 across clinically relevant drug concentrations [37]. In addition, preclinical data found the luminal subtype, elevated cyclin D1 (CCND1) and Rb protein expression and reduced p16 expression as associated with sensitivity to palbociclib [37]. AR+ TNBC expresses a luminal profile. Palbociclib has been shown to reduce growth of MDA-MB-453 breast cancer cells via reduced Rb phosphorylation; it inhibits the incorporation of thymidine into the DNA of RB-positive breast cancer cells [37]. In addition, AR+ TNBC has intact Rb protein, the target of palbociclib activity [37]. Based on these collective data, a phase I/II evaluating the combination of bicalutamide and palboiclib in women with AR+ TNBC is currently enrolling patients (NCT02605486) [38].

Dual PI3K and AR inhibition—The PI3K/AKT/mTOR signaling pathway is integral in cell growth and survival and has been implicated in breast cancer development. Activating mutations in PI3KCA have been described in association with androgen receptor AR positivity in breast tumors [25, 39, 40]. Association between AR and pAKT, a known downstream target of this pathway, has also been reported ($P=0.003$)[41]. The interaction between AR and the PI3K signaling pathway has been a particular focus of investigation within the AR+ TNBC population as a potential therapeutic target. Lehmann et al. demonstrated significantly increased sensitivity of LAR cell lines, which are frequently associated with PIK3CA mutations, to NVP-BEZ235 (a dual PI3K/mTOR inhibitor) when compared to the basal-like subtype, ($IC_{50}=37$ nM vs $IC_{50}=116$ nM, $P=0.01$)[25]. These preclinical findings have been translated into a multicenter phase I/II trial of enzalutamide in combination with taselisib for the treatment of patients with advanced AR+ TNBC through the TBCRC (NCT02457910).

Conclusion

The characterization of “TNBC” as a unique breast cancer subtype is a relatively recent development. In the intervening years, advances in molecular profiling have led to the categorization of TNBC into multiple reproducible subgroups distinguished by distinct gene signatures. Clinically the use of the term TNBC highlights the shortcomings of this classification as this cohort is defined largely by what it lacks rather than by molecular subclassification. Cost and complexity of gene expression profiling limit its everyday use in the clinical setting. However these innovations have led to the identification of molecular targets and continue to inform the development of novel therapeutic agents. Several groups have described a subtype of breast cancer which demonstrates increased AR expression and low expression of ER, PR, and HER2. And now preclinical and clinical data have confirmed the potential for androgen blockade as a treatment option for AR+ TNBC. Ongoing research in this area has the potential to ultimately allow a greater number of patients with breast cancer the option of safe and effective endocrine therapy, delaying the need for cytotoxic chemotherapy.

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Table 1

Targeting the androgen signaling pathway in TNBC (select list of active trials)

Stage	Clinical Trial	Phase	Agent	Mechanism of Action	Objectives
Early	NCT02750358	II (Adjuvant)	Enzalutamide	AR inhibitor	Primary: To evaluate the treatment discontinuation rate of enzalutamide for 1 year due to toxicity, withdrawal of consent or other events related to tolerability. Secondary: DFS, OS, safety and tolerability
	NCT02689427	II (Neoadjuvant)	Enzalutamide + paclitaxel	AR inhibitor + antimicrotubule agent	Primary: pCR, RCP-1 Secondary: PFS
	NCT02676986	II (Window of opportunity)	Enzalutamide	AR inhibitor	Primary: Determine the individual anti- proliferative response (RR Ki67) Secondary: -Determine the geometric mean change in Ki67 expression at the end of study treatment (Mean Ki67) -Determine the individual end-of treatment anti- proliferative response (RRKi67-Post) -Determine the geometric mean change in Caspase-3 between end of study treatment and pre- treatment tumor samples -Determine the individual apoptotic response (RR Caspase-3) -Measure the plasma levels of circulating hormones in blood samples collected prior to and at the end of study treatment
Metastatic					
	NCT02580448	I/II	Seviteronel	Dual AR inhibitor + lyase-selective CYP17 inhibitor	Primary Phase I: MTD Phase II: CBR at 16 weeks Secondary: PK assessment, ORR, PFS, OS, safety and tolerability, correlative studies
	NCT02605486	I/II	Bicalutamide + palbociclib	AR inhibitor	Primary Phase I: RP2D Phase II: PFS at 6 months Secondary: ORR, CBR at 24 weeks, PFS, safety and tolerability
	NCT02457910	I/II	Enzalutamide+ taselisib	AR inhibitor + PI3K inhibitor	Primary Phase I: MTD Phase II: CBR at 16 weeks Secondary: overall PFS, ORR, PK assessment, correlative studies
	NCT02368691	II	GTx-024	Selective androgen receptor modulator (SARM)	Primary: CBR at 16 weeks Secondary: CBR at 24 weeks, ORR, best overall response, PFS, duration of response, PK assessment, safety and tolerability
	NCT01990209	II	Orteronel	17,20-lyase inhibitor	Primary: RR, DCR Secondary: PFS, OS, safety and tolerability, measurement of changes in serum hormone levels, assess loss of expression of PTEN and mutations in PIK3CA gene,
	Pending	III	Enzalutamide vs enzalutamide + paclitaxel vs placebo + paclitaxel	AR inhibitor +/- antimicrotubule agent	Primary: PFS

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Abbreviations: CBR, clinical benefit rate; CR, complete response; DCR, disease control rate; DFS, disease-free survival; MTD, maximum tolerated dose; ORR, objective response rate; OS, overall survival; pCR, pathologic complete response; PK, pharmacokinetic; PR, partial response; PFS, progression-free survival; RCB-I, minimal residual disease; RP2D, recommended phase II dose; RR, response rate