Anti-androgen therapy in triple-negative breast cancer

Valerie N. Barton, Michael A. Gordon, Jennifer K. Richer and Anthony Elias

Ther Adv Med Oncol

2016, Vol. 8(4) 305–308

DOI: 10.1177/1758834016646735

Correspondence to:
Anthony Elias, ADE, MD
Division of Medical Oncology, University of Colorado School of Medicine, Anschutz Medical Campus, ACP 5310, MS 8117 1665 Aurora Court, Aurora, CO 80045, USA
Anthony.Elias@ucdenver.edu

Valerie N. Barton, PhD
Michael A. Gordon, PhD
Jennifer K. Richer, PhD
Department of Pathology, University of Colorado School of Medicine, Anschutz Medical Campus, Aurora, CO, USA

Introduction

Inhibition of the androgen receptor (AR) represents the most important therapeutic target in prostate cancer. Although AR is expressed in 77% of all breast cancers (BCs), even more than estrogen receptors (ERs), its role in BC growth and progression remains indefinite [Guedj *et al.* 2012]. AR expression is associated with somewhat more indolent BC [Lehmann *et al.* 2011; Liedtke *et al.* 2008; Cochrane *et al.* 2014]. The drug development pipeline of AR-targeted therapeutics in prostate cancer is facilitating the evaluation of AR signaling inhibition in triple-negative breast cancer (TNBC): including bicalutamide, a nonsteroidal partial agonist; enzalutamide, an inhibitor of nuclear localization of AR; and VT-464, a dual inhibitor of CYP17 and AR. Given the controversy in the role of AR, other ongoing or completed trials are testing dehydroepiandrosterone (DHEA) or 4-OH testosterone (see Table 1).

Preclinical justification for anti-androgen therapies in breast cancer

Gene expression profiling of BC suggests a significant functional role for AR in multiple subtypes of BC [Guedj *et al.* 2012; Lehmann *et al.* 2011]. While AR is expressed to varying degrees across all BC subtypes, preclinical modeling suggests that its functional role in disease progression is subtype-specific. Gene expression profiling of TNBC has revealed a number of potential subtypes within TNBC, including basal-like 1, basal-like 2, immunomodulatory, mesenchymal-like, mesenchymal stem-like, and luminal AR (LAR) [Lehmann *et al.* 2011], although these subtypes do not yet dictate individualized treatment with specific targeted agents to date. Although ER expression is absent, the LAR subtype is characterized by AR signaling with a gene expression pattern similar to luminal BC. Patients with LAR tumors are more slowly growing when metastatic, however they have decreased relapse-free survival in the adjuvant setting relative to other TNBC subtypes [Cochrane *et al.* 2014], perhaps due to lower chemotherapy sensitivity. LAR cell line models are sensitive to the AR partial antagonist bicalutamide [Lehmann *et al.* 2011], and are even more sensitive to the next-generation AR inhibitor enzalutamide [Cochrane *et al.* 2014].

AR is expressed in 12–55% of cases of TNBC [Barton *et al.* 2015; Collins *et al.* 2011; Gucalp *et al.* 2013; Thike *et al.* 2014; Traina *et al.* 2015]. Some of the variability in frequency of expression between studies is due to different anti-AR antibodies used and to different assay cutoffs (1% *versus* 10%). Preclinically, BC expressing as little as 1% AR may respond to enzalutamide, although higher levels may be associated with greater response [Barton *et al.* 2015]. Optimal assay for response to AR inhibitors in clinic is as yet unknown. Although the LAR subtype of TNBC is AR enriched, other TNBC subtypes also express AR, and have responded to AR inhibition using preclinical models [Barton *et al.* 2015]. In TNBC models, AR appears to regulate amphiregulin (AREG), an epidermal growth factor receptor (EGFR) ligand, which when secreted could potentially support even AR negative tumor cells [Barton *et al.* 2015].

Phosphoinositide 3-kinase (PI3K3) activation through loss of phosphatase and tensin homolog (PTEN) or mutation of PIK3CA is common in TNBC [Shah *et al.* 2012; Kriegsmann *et al.* 2014], and is associated with increased AR levels in BC [Gonzalez-Angulo *et al.* 2009]. The combination of bicalutamide and the PI3K inhibitors pictilisib and apitolisib showed additive efficacy in PI3K-mutant TNBC cells *in vitro* and *in vivo* [Lehmann *et al.* 2014]. Enzalutamide plus everolimus appeared to be synergistic in multiple *in vitro* preclinical models of BC, including TNBC [Gordon *et al.* 2014].

Table 1. Clinical trials of AR therapies in breast cancer.

ClinicalTrials.gov identifier	Phase	Patient population	Treatment
NCT01889238	II	Advanced AR+ TNBC	Enza
NCT02457910	I/II	Postmenopausal AR+, metastatic TNBC	Enza +/– taselisib
NCT02348281	II	Postmenopausal AR+, metastatic TNBC	Bicalutamide
NCT02067741	I	Postmenopausal metastatic or locally advanced, endocrine responsive-Her2- and TN-AR+	CR1447 (4-OH-testosterone)
NCT02000375	II	Postmenopausal pretreated metastatic, AR+	DHEA
NCT02000375	II	Postmenopausal pretreated metastatic, AR+	DHEA
NCT02368691	II	Advanced AR+ TNBC	GTx-024
NCT02580448	I/II	Advanced AR+ TNBC; ER+/Her2- BC	VT-464
NCT02605486	II	Advanced AR+ BC	Bicalutamide + palbociclib
NCT02689427	IIB	Advanced AR+ BC	Taxol +/– enzalutamide

AR, androgen receptor; BC, breast cancer; DHEA,; ER, estrogen receptors; Her2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer; VT,

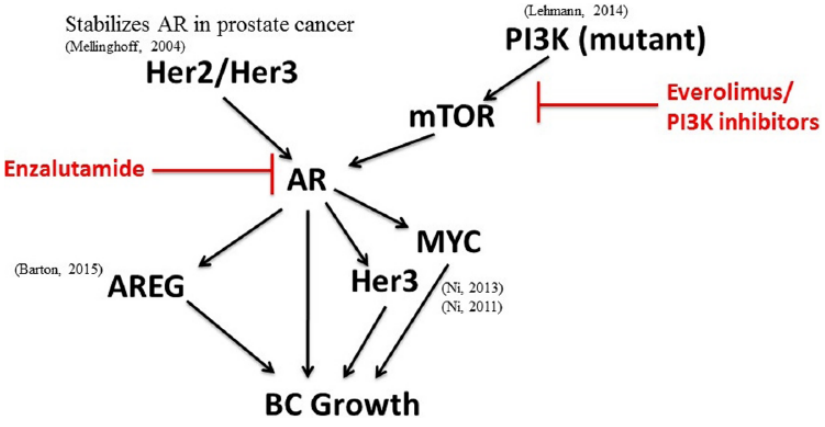

Figure 1. AR signaling integration in TNBC.
Targeted therapies are highlighted in red. AR, androgen receptor; AREG, amphiregulin; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; MYC, myelocytomatosis viral oncogene homolog; Her2, human epidermal growth factor receptor 2; Her3, human epidermal growth factor receptor 3; BC, breast cancer.

Clinical trials of anti-AR therapies in TNBC

Promising preclinical modeling of AR inhibition in TNBC has led to evaluation in the clinic. Interim results suggest that enzalutamide in particular provides significant clinical benefit for AR+ TNBC. A summary of trials is listed in Table 1.

Of 424 patients with ER/progesterone receptor (PR) negative metastatic breast cancer eligible for testing were screened by immunohistochemistry (IHC) for AR using a Dako antibody (AR441), 51 (12%) had >10% AR staining in archived tissues. Ultimately 26 patients with advanced AR+ TNBC (four had ER/PR 1–10%) were enrolled into a phase II trial of bicalutamide 150 mg po daily run by Memorial Sloan Kettering Cancer Center (MSKCC, New York, NY, USA) and the Translational Breast Cancer Research Consortium (TBCRC). The patients had a median age of 66 years, performance status (PS) of 0, and a median of 1 (0–8) prior lines of chemotherapy for metastatic disease. Median progression-free survival (PFS) was 12 weeks (95% CI: 11, 23). A total of five patients (ER 0–3%, PR negative) had stable

disease with a clinical benefit rate (CBR) at 24 weeks of 19% (95% CI: 7, 39), including one patient on therapy for 57+ months [Gucalp *et al.* 2013]. No partial responses (PRs) or complete responses (CRs) were observed. The most common possibly drug-related toxicities included grade 1/2 fatigue, hot flashes, limb edema, and transaminitis.

A phase II trial of single-agent enzalutamide in advanced AR+ TNBC has been completed [Traina *et al.* 2015]. In this trial, AR positivity was defined as at least 1% nuclear staining by IHC (using a Ventana antibody). Patients with advanced AR+ TNBC with any number of prior therapies were eligible. Because of a possible risk for seizures with enzalutamide, no brain metastases were allowed. The primary endpoint was CBR at 16 weeks. The study was designed as a Simon two-stage trial powered to have an 85% power to detect a true CBR16 of $\leq$8% *versus* $\geq$20% with a 1-sided alpha of 5%. Of 165 patients screened, 118 (72%) (intent-to-treat (ITT) population) were AR+, of whom 89 had AR staining $\geq$10%. Of the patients with AR IHC $\geq$ 10% and who had a post-baseline tumor assessment, 75 patients constituted the 'evaluable population'. Median age was 57 years and median prior therapy for advanced TNBC was 1 (0–8). At the time of presentation at the 2015 ASCO meeting, 11 (9%) were still on treatment. The CBR16 was 25% (95% CI: 17, 33), CBR24 20% (95% CI: 14, 29), CR/PR 6%, and median PFS 13 weeks for the ITT patient population. Most of the benefit was concentrated in the 'evaluable population'. The predominant toxicities were fatigue (40% (5% G3)), nausea (32%), decreased appetite (19%). Grade 3 toxicities were observed in 10%. From 178 tissues (AR+ in 140, and AR- in 38), 521 genes were significantly different between the AR+ and AR- tissues. A proprietary androgen-driven gene signature called PREDICT AR was created from gene expression profiling, and patients whose tumors were positive for this signature had increased progression-free survival compared to those without an androgen-driven gene signature (32 *versus* 9 weeks).

Phase Ib/II trials of enzalutamide with or without the PI3K inhibitor taselisib, and VT-464 in TNBC are ongoing, with results not yet reported (ClinicalTrials.gov identifiers: NCT02457910, NCT02580448). VT-464 is a combination inhibitor of AR as well as CYP17, and therefore it dramatically decreases the ligands estradiol and testosterone, without requiring steroid replacement.

Conclusion

The role of the androgen receptor in BC biology remains controversial, but based on preclinical studies and new clinical trials, inhibition of AR signaling appears to be a viable therapeutic target. At this point, there is some evidence that AR inhibition has clinical benefit in TNBC (some more prolonged stable disease, occasional CR/PR), however the CBR at 16 and 24 weeks may be confounded by the observation that AR+ TNBC (particular with luminal gene patterns) may be more indolent biologically. The correlation between potential benefit and AR expression by IHC is not strong, although the study by Traina and colleagues would suggest that benefit may be more likely in tumors with >10% AR nuclear expression [Traina *et al.* 2015]. The PREDICT AR test might enhance the signal because it includes gene expression downstream of AR, and thus would indicate tumors in which the AR pathway is activated. However, because this test is proprietary, it is not possible to analyze this further.

AR inhibition alone is well tolerated and may be useful to patients with TNBC, as the toxicity is significantly less than that of chemotherapy. However, it is likely that AR inhibition will be combined with other agents. Preclinical data would support combinations with paclitaxel and other chemotherapy agents [Gordon *et al.* 2014], combination with mTOR inhibitors [Gordon *et al.* 2014], combination with EGFR and other ErbB inhibitors [Barton *et al.* 2015], combination with PIK3 inhibitors [Kriegsmann *et al.* 2014], and combinations with anti-PDL1 antibodies [Tung *et al.* 2015]. Randomized trials will be needed to establish the clinical utility of AR inhibitors. Validated predictive biomarkers will be critical to select appropriate patients for AR inhibition.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. DOD BCRP Clinical Translational Award BC120183 to JKR and ADE.

Conflict of interest statement

The author(s) declared that there is no conflict of interest.

References

Barton, V., D'Amato, N., Gordon, M., Lind, H., Spoelstra, N., Babbs, B. *et al.* (2015) Multiple molecular subtypes of triple-negative breast cancer critically rely on androgen receptor and respond to enzalutamide in vivo. *Mol Cancer Ther* 14: 769–778.

Cochrane, D., Bernales, S., Jacobsen, B., Cittelly, D., Howe, E., D'Amato, N. *et al.* (2014) Role of the androgen receptor in breast cancer and preclinical analysis of enzalutamide. *Breast Cancer Res* 16: R7.

Collins, L., Cole, K., Marotti, J., Hu, R., Schnitt, S. and Tamimi, R. (2011) Androgen receptor expression in breast cancer in relation to molecular phenotype: results from the Nurses' Health Study. *Modern Pathol* 24: 924–931.

Gonzalez-Angulo, A., Stemke-Hale, K., Palla, S., Carey, M., Agarwal, R., Meric-Berstam, F. *et al.* (2009) Androgen receptor levels and association with PIK3CA mutations and prognosis in breast cancer. *Clin Cancer Res* 15: 2472–2478.

Gordon, M., D'Amato, N., Gu, H., Wong, D., Elias, A. and Richer, J. (2014) Targeting multiple pathways in breast cancer: Androgen receptor, HER2, and mTOR. *Cancer Res* 75: P6-03-07.

Gucalp, A., Tolaney, S., Isakoff, S., Ingle, J., Liu, M., Carey, L. *et al.* (2013) Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic Breast Cancer. *Clin Cancer Res* 19: 5505–5512.

Guedj, M., Marisa, L., de Reynies, A., Orsetti, B., Schiappa, R., Bibeau, F. *et al.* (2012) A refined molecular taxonomy of breast cancer. *Oncogene* 31: 1196–1206.

Kriegsmann, M., Endris, V., Wolf, T., Pfarr, N., Stenzinger, A., Loibl, S. *et al.* (2014) Mutational profiles in triple-negative breast cancer defined by ultradeep multigene sequencing show high rates of PI3K pathway alterations and clinically relevant entity subgroup specific differences. *Oncotarget* 5: 9952–9965.

Lehmann, B., Bauer, J., Chen, X., Sanders, M., Chakravarthy, A., Shyr, Y. *et al.* (2011) Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest* 121: 2750–2767.

Lehmann, B., Bauer, J., Schafer, J., Pendleton, C., Tang, L., Johnson, K. *et al.* (2014) PIK3CA mutations in androgen receptor-positive triple-negative breast cancer confer sensitivity to the combination of PI3K and androgen receptor inhibitors. *Breast Cancer Res* 16: 406.

Liedtke, C., Mazouni, C., Hess, K., André, F., Tordai, A., Mejia, J. *et al.* (2008) Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. *J Clin Oncol* 26: 1275–1281.

Mellinghoff, I., Vivanco, I., Kwon, A., Tran, C., Wongvipat, J. and Sawyers, C. (2004) HER2/neu kinase-dependent modulation of androgen receptor function through effects on DNA binding and stability. *Cancer Cell* 6: 517–527.

Ni, M., Chen, Y., Fei, T., Li, D., Lim, E., Liu, X. *et al.* (2013) Amplitude modulation of androgen signaling by c-MYC. *Genes Dev* 27: 734–748.

Ni, M., Chen, Y., Lim, E., Wimberly, H., Bailey, S., Imai, Y. *et al.* (2011) Targeting androgen receptor in estrogen receptor-negative breast cancer. *Cancer Cell* 20: 119–131.

Shah, S., Roth, A., Goya, R., Oloumi, A., Ha, G., Zhao, Y. *et al.* (2012) The clonal and mutational evolution spectrum of primary triple-negative breast cancers. *Nature* 486: 395–399.

Thike, A., Yong-Zheng Chong, L., Cheok, P., Li, H., Wai-Cheong Yip, G., Huat Bay, B. *et al.* (2014) Loss of androgen receptor expression predicts early recurrence in triple-negative and basal-like breast cancer. *Modern Pathol* 27: 352–360.

Traina, T., Miller, K., Yardley, D., O'Shaughnessy, J., Cortes, J., Awada, A. *et al.* (2015) Results from a phase 2 study of enzalutamide (ENZA), an androgen receptor (AR) inhibitor, in advanced AR+ triple-negative breast cancer (TNBC). *J Clin Oncol* 33: 1003.

Tung, N., Garber, J., Torous, V., Hacker, M., Freeman, G., Rodig, S. *et al.* (2015) Prevalence and predictors of androgen receptor (AR) and programmed death-ligand 1 (PD-L1) expression in BRCA1-associated and sporadic triple-negative breast cancer (TNBC). *J Clin Oncol* 33: 1005.