Targeting the androgen receptor in prostate and breast cancer: several new agents in development

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Abstract

Prostate cancer (PCa) and breast cancer (BCa) share similarities as hormone-sensitive cancers with a wide heterogeneity of both phenotype and biology. The androgen receptor (AR) is a hormone receptor involved in both benign and malignant processes. Targeting androgen synthesis and the AR pathway has been and remains central to PCa therapy. Recently, there has been increased interest in the role of the AR in BCa development and growth, with results indicating AR co-expression with estrogen, progesterone, and human epidermal growth factor receptors, across all intrinsic subtypes of BCa. Targeting the AR axis is an evolving field with novel therapies in development which may ultimately be applicable to both tumor types. In this review, we offer an overview of available agents which target the AR axis in both PCa and BCa and provide insights into the novel drugs in development for targeting this signaling pathway.

Key Words
androgen receptor
hormone receptor
breast cancer
prostate cancer

Introduction

The androgen receptor (AR) is a member of the steroid-hormone family involved in the regulation of normal growth and development within a broad array of target organs. It is a member of the family of steroid receptors that includes the glucocorticoid, progesterone, and mineralocorticoid receptors. AR transcription is age- and cell-type-dependent, modulated by the presence of circulating androgens (Gelmann 2002). The eight-exon gene, located on the X chromosome (Xq11–12), encodes a 110 kDa protein with four functionally distinct regions (Fig. 1; Gelmann 2002). The N-terminal domain (exon 1) is involved in transcriptional activation involving interaction with co- regulatory proteins. The DNA-binding domain (DBD) (exons 2 + 3) is a highly conserved region, which folds into a two-zinc-finger motif critical to DNA binding. The ligand binding domain (LBD) on the C-terminus (exons 4–8) is responsible for the binding of androgens. The hinge region links the DBD and LBD, and is responsible for nuclear localization (Chang et al. 1995, Gelmann 2002, Lee & Chang 2003, Heinlein & Chang 2004, Claessens et al. 2008).

The normal function of the AR is dependent on ligand binding and interaction with co-activators and chaperone proteins. In the absence of ligand, the AR is present in the cytoplasm bound to heat shock proteins (i.e., hsp90) and other co-chaperones retaining an inactive conformation.
Upon exposure and binding to androgen (testosterone or dihydrotestosterone (DHT)), phosphorylation and conformational changes occur. Binding of ligand to the hormone-binding site leads to the formation of a co-activator-binding site (AF-2 site) and reconfiguration of the components of the helical protein structure (H3, H4, and H12) (Osguthorpe & Hagler 2011). Transcription of target genes is prompted by the dissociation of chaperone proteins, receptor dimerization, and exposure of the nuclear localizing signal which leads the two zinc fingers of the DBD to bind to the genomic androgen response elements (Gelman 2002, Lee & Chang 2003, Claessens et al. 2008).

In normal tissue, androgen-responsive genes are important for normal prostate architecture, homeostasis, and physiological function. In prostate cancer (PCa) cells, expression of these genes leads to the proliferation and survival of tumor cells. In breast tissue, the relationship is less clear. In normal breast tissue, androgens are involved in inhibiting breast development (Dimitrakakis & Bondy 2009). In breast cancer (BCa), androgens have been shown to induce proliferative changes in breast tissue and promote growth of some BCa cell lines (Xie et al. 1999, Wong & Xie 2001).

Prostate cancer
The role of the AR in PCa

Since 1941 when Huggins and Hodges first demonstrated that hormonal manipulation could result in antitumor activity in PCa (Huggins et al. 1941), androgen deprivation therapy (ADT) has been an essential component of the treatment of advanced disease. However, as the disease evolves, castration-sensitive PCa initially responding to ADT eventually develops mechanisms of resistance leading to PCa growth and devolvement to a castration-resistant disease state. However, even in castration-resistant PCa (CRPC), the AR continues to signal and drive disease growth. This fact has formed the biologic basis for the development of two novel agents, enzalutamide and abiraterone acetate.

AR signaling through CRPC

Medical or surgical castrating therapy is highly effective in over 80% of patients with newly diagnosed castration-sensitive PCa (Crawford et al. 1989). However, over time, PCa develops resistance mechanisms that are generally manifested first by an increase in prostate-specific antigen (PSA) despite androgen-lowering therapies. This phase of the disease is termed CRPC (Scher & Heller 2000). The mechanisms driving CRPC include upregulation of alternative androgen production pathways (Titus et al. 2005, Mostaghel et al. 2007), AR gene amplification or protein overexpression (Visakorpi et al. 1995, Bubendorf et al. 1999, Haapala et al. 2007), mutations within the LBD of the AR (Marcelli et al. 2000, Taplin et al. 2003), and activation of other signal transduction pathways (Carver et al. 2011).

The CRPC cells have been shown to maintain intratumoral levels of testosterone even in the setting of androgen-lowering agents (Titus et al. 2005, Mostaghel et al. 2007). Orchiectomy or luteinizing hormone-releasing hormone agonists or antagonists have little to no effect on adrenal or intratumoral androgen production. Specifically, in the setting of medical castration, adrenal androgens such as DHEA and androstenedione are
relatively unaffected. In addition, results of studies comparing metastatic CRPC (mCRPC) to primary tumor samples indicated that within the mCRPC tumor cells, there was upregulation of enzymes involved in androgen synthesis, most notably those catalyzed by members of the cytochrome P450 family (Holzbeierlein et al. 2004, Stanbrough et al. 2006, Locke et al. 2008, Montgomery et al. 2008). AR gene amplification or protein overexpression is believed to be one of the key mechanisms in CRPC (Visakorpi et al. 1995, Bubendorf et al. 1999, Haapala et al. 2007). Unlike primary untreated PCa samples where AR overexpression is rare (<2%), locally recurrent PCa and metastatic sites in CRPC display AR gene amplification in 23% and 22% of samples respectively (Bubendorf et al. 1999). Circulating tumor cells taken from patients with mCRPC have also been shown to have AR overexpression in 30–60% of the samples analyzed (Bubendorf et al. 1999, Attard et al. 2009, Leversha et al. 2009). It is believed that AR overexpression leads to increased sensitivity of the AR toward castrate levels of testosterone, allowing the AR axis to continue to drive growth and proliferation even in the castrate state (Visakorpi et al. 1995, Waltering et al. 2009). Results obtained using in vitro models have indicated that AR overexpression results in the conversion of the first-generation AR antagonist bicalutamide to an agonist by altering the recruitment of AR co-activator and co-repressor molecules (Chen et al. 2004).

Mutations in the AR in early-stage PCa are present, but relatively rare (Marcelli et al. 2000). However, as the tumor progresses to a castration-resistant state, mutations within the AR can be seen in more than 10% of patients who have received treatment with first-generation antiandrogens (Tatling et al. 2003). Currently, over 100 somatic mutations in the AR have been identified, and the most clinically relevant are thought to alter co-factor binding and ligand specificity. These mutations have been described as enabling alternative steroidal molecules, including progestosterone and AR antagonists, to activate the AR pathway (Culig et al. 1993, Zhao et al. 2000).

Another potential mechanism leading to CRPC is the activation of other signal transduction pathways which bypass or interact with the AR axis. The phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway is a commonly identified pathway that has shown an association with the AR in PCa (Carver et al. 2011, Thomas et al. 2013). Results obtained using in vivo models have revealed that the activation of one pathway leads to the downregulation of the other due to a reciprocal feedback mechanism, and inhibition of both pathways has led to near-complete cancer regression in a Pten-deficient murine PCa model (Carver et al. 2011). Using an in vitro model, it has also been shown that combining the AKT inhibitor AZD5363 with the AR antagonist bicalutamide resulted in synergistic inhibition of cell growth and induction of cell death (Thomas et al. 2013).

First-generation antiandrogens

Bicalutamide, nilutamide, and flutamide are first-generation or conventional nonsteroidal antiandrogens that bind to the LBD of the AR with a relatively low affinity compared with androgens. Historically, the indication for first-generation antiandrogens was to prevent a flare phenomenon or a transient rise of a patient’s testosterone level after the initiation of a gonadotropin-releasing hormone (GNRH) analog (Kuhn et al. 1989). Another commonly used indication for first-generation antiandrogens, which remains controversial, is their use with a GNRH agonist, a strategy called combined androgen blockade (CAB). Multiple large phase III clinical trials, as well as multiple large meta-analyses, have compared the efficacy of CAB to a GNRH agonist alone (Prostate Cancer Trialists’ Collaborative Group 1995, 2000, Bennett et al. 1999, Samson et al. 2002). The National Comprehensive Cancer Network (NCCN) PCa guidelines version 1.2015 (National Comprehensive Cancer Network 2015) acknowledge that CAB provides little to no benefit over ADT alone in patients with metastatic disease, and do not recommend antiandrogen monotherapy in that it appears to be less effective than surgical or medical castration. However, the NCCN guidelines also cite a meta-analysis that showed a 5–20% improvement in overall survival (OS) with CAB compared with GNRH agonist alone (Samson et al. 2002).

One limitation of the long-term use of first-generation antiandrogens is the well-documented, AR-driven, antagonist-to-agonist potential. Identified mutations within the LBD at codon 741 or codons 874 and 877 result in bicalutamide or flutamide, respectively, being transformed from a weak antagonist to an agonist (Tan et al. 1997, Hara et al. 2003). The agonist potential of first-generation antiandrogens is manifested by a paradoxical decline in PSA upon antiandrogen discontinuation, a phenomenon known as antiandrogen withdrawal (AAWD) (Kelly & Scher 1993, Small & Carroll 1994).

Two large phase III clinical trials conducted by the Southwest Oncology Group (SWOG) and the Cancer and Leukemia Group B (CALGB), respectively, measured the incidence of AAWD in first-generation antiandrogens. SWOG 9426 evaluated 210 patients with progressive PCa...
for a median follow-up of 5.0 years. Sixty-four percent of the patients were on flutamide, 32% on bicalutamide, and 3% on nilutamide. It was found that 21% of patients had a PSA decline ≥50% with a median progression-free survival (PFS) of 3 months after stopping the first-generation antiandrogen. Furthermore, 19% of patients had a 1-year or greater progression-free interval, indicating an overall durable response to stopping treatment (Sartor et al. 2008).

CALGB 9583 evaluated the therapeutic effect of AAWD versus AAWD with ketoconazole therapy in patients with CRPC. This study found an 11% rate of PSA response in the AAWD-alone arm versus a 27% rate of response with the addition of ketoconazole. However, there was no difference in OS between arms (Small et al. 2004).

**A second-generation antiandrogen: enzalutamide**

Enzalutamide (formerly MDV3100) is an oral nonsteroidal antagonist of the AR. The preclinical development phase for MDV3100 used multiple cell lines. For example, the LNCaP/AR cell line was constructed to contain higher factors including brain metastasis (Scher et al. 2012, Beer et al. 2014). However, given the concern regarding seizure activity, a phase IV multicenter, single-arm, open-label clinical trial is ongoing to evaluate the risk of seizure among patients with mCRPC at high risk for seizure activity (UPWARD trial (NCT01977651)).

PREVAIL evaluated chemotherapy-naïve men with mCRPC in a 1:1 randomization to enzalutamide 160 mg/day or placebo. The primary endpoints of the trial were rPFS and OS. A total of 1717 patients were evaluated (872 enzalutamide, 845 placebo). At 12-month follow-up, the rate of rPFS was 65% in the enzalutamide arm versus 14% in the placebo arm (HR 0.19; 95% CI 0.15–0.23; P<0.001). At the time of the interim analysis, 72% of patients in the enzalutamide arm were alive compared with 63% in the placebo arm, which correlated to a significant 29% reduction in the risk of death in the enzalutamide arm (HR 0.71; 95% CI 0.50–0.98; P<0.001). In addition, all prespecified secondary endpoints favored the treatment arm, including time until the start of chemotherapy, time to first SRE, complete or partial soft-tissue response, time to PSA progression, and rate of ≥50% PSA decline. Fatigue and hypertension were the most common grade 3 or higher AEs. Two seizure events occurred on trial, one in each treatment arm (Beer et al. 2014).

Enzalutamide is now being investigated in a variety of combinatorial trials. These include a phase III clinical trial in first-line mCRPC comparing the combination of enzalutamide plus abiraterone acetate and prednisone to enzalutamide alone (led by the Alliance for Clinical Trials
in Oncology (NCT01949337)). Enzalutamide is also being compared with placebo in patients with non-metastatic CRPC in the PROSPER trial (NCT02003924).

**Mechanism of resistance to enzalutamide** Notwithstanding the promising results from the AFFIRM and PREVAIL trials, most men on enzalutamide will develop progressive disease and therefore identification of resistance mechanisms is critical (Scher et al. 2012, Beer et al. 2014). A mutation within the LBD of the AR, F876L, has been shown to be a driver of enzalutamide resistance (Balbas et al. 2013). Similar to the LBD mutations that result in resistance to first-generation antiandrogens, F876L has been shown to convert enzalutamide, as well as ARN-509, another second-generation AR antagonist, from an antagonist to an agonist (Balbas et al. 2013).

Upregulation of the glucocorticoid receptor (GR) has also been identified as a mechanism of resistance toward enzalutamide. Acute AR inhibition leads to GR upregulation and when the GR, a nuclear receptor in the same family (NR3C) as the AR, binds to its ligand (dexamethasone), an enzalutamide-resistant phenotype is maintained. However, when a GR antagonist is introduced, enzalutamide sensitivity is reestablished, indicating an important mechanistic relationship between the AR and the GR in enzalutamide resistance (Balbas et al. 2013). In addition, results of a post hoc analysis of the AFFIRM trial indicated that patients who were on corticosteroids during the study had a reduction in OS and higher rates of grade 3/4 toxicity independent of their treatment (Scher et al. 2013).

The PI3K/AKT/mTOR signaling pathway has also been shown to be a potential mechanism of resistance toward enzalutamide. As stated previously, preclinical models have revealed that the AR and the PI3K pathways cross-regulate each other through a reciprocal feedback mechanism and there are currently ongoing clinical trials evaluating the safety and efficacy of inhibiting both pathways (Table 1; Carver et al. 2011).

Finally, the AR splice variant (AR-V), specifically AR-V7, has recently been shown to be a potential mechanism of resistance and a predictive marker of response to enzalutamide in both preclinical and clinical models. One study evaluated 31 men treated with enzalutamide, of whom 38.7% had a detectable AR-V7 measured using circulating tumor cells. Compared with subjects without detectable AR-V7, patients with AR-V7 had inferior PSA response rates (0% versus 53%; P=0.004), time to PSA progression (median, 1.4 months versus 6.0 months; P<0.001), clinical and rPFS (median, 2.1 months versus 6.1 months; P<0.001), and OS (median, 5.5 months versus not reached; P=0.002) (Antonarakis et al. 2014). Preclinical data have indicated that AR-V expression is increased in a castrate state and potentially suppressed by testosterone. It is believed by some that the AR-V is an acute biochemical response to castration rather than a mutation associated with a gain-of-function or clonal expansion to enzalutamide. These findings will need to be validated clinically but could provide insights into the AR-V resistance pattern (Watson et al. 2010). Further trials are ongoing to validate the AR-V7 assay as a potential predictive biomarker for AR-directed therapy in patients with CRPC.

**Table 1** Novel agents targeting the AR axis: early-phase trials and future directions

<table>
<thead>
<tr>
<th>Agent</th>
<th>Phase</th>
<th>Population</th>
<th>Notes</th>
<th>References/clinicaltrials.gov</th>
</tr>
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<tbody>
<tr>
<td>AR-targeted</td>
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<tr>
<td>AZD3514</td>
<td></td>
<td>mCRPC</td>
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<td>NCT01162395 (active, not recruiting) NCT02144051 (recruiting)</td>
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<td>AZD3514</td>
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<td>Combination trials</td>
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<tr>
<td>ARN-509 + AA</td>
<td></td>
<td>mCRPC</td>
<td>AR targeting</td>
<td>NCT02123758 (recruiting)</td>
</tr>
<tr>
<td>BEZ235 or BKM120 + AA</td>
<td></td>
<td>mCRPC</td>
<td>Pan class PI3K inhibitor</td>
<td>NCT01634061 (active, not recruiting)</td>
</tr>
<tr>
<td>BMK120 + AA</td>
<td></td>
<td>mCRPC</td>
<td>Pan class PI3K inhibitor</td>
<td>NCT01741753 (recruiting)</td>
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<tr>
<td>GDC-0068 or GDC-0980 + AA</td>
<td></td>
<td>mCRPC</td>
<td>Pan-Akt</td>
<td>NCT01485861 (recruiting)</td>
</tr>
<tr>
<td>Dasatinib or sunitinib + AA</td>
<td></td>
<td>mCRPC</td>
<td>ABL, SRC, or VEGFR, EGFR tyrosine kinase inhibitor</td>
<td>NCT01254864 (active, not recruiting)</td>
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<tr>
<td>Alisertib + AA</td>
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<td>mCRPC</td>
<td>Small-molecule inhibitor of serine/threonine protein kinase, aurora A kinase</td>
<td>NCT01848067 (active, not recruiting)</td>
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<td>Tivozanib + enzalutamide</td>
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<td>mCRPC</td>
<td>Antisense to clusterin mTOR inhibitor</td>
<td>NCT01885949 (recruiting) NCT02106507 (recruiting)</td>
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<tr>
<td>Temsirolimus + ARN-509</td>
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<td>mCRPC</td>
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AR, androgen receptor; mCRPC, metastatic castration-resistant prostate cancer; AA, abiraterone acetate; PI3K, phosphatidylinositol 3-kinase; VEGFR, vascular endothelial growth factor receptor; EGFR, epidermal growth factor receptor; mTOR, mammalian target of rapamycin.
An androgen biosynthesis inhibitor: abiraterone acetate

Abiraterone acetate (AA) (formerly known as CB7630), a prodrug of abiraterone, is a potent, orally available, selective inhibitor of both 17α-hydroxylase/c17,20-lyase which targets adrenal and tumor intracrine androgen biosynthesis. Preclinical and early-phase clinical studies displayed promising efficacy with AA for patients with mCRPC; these led to two large phase III clinical trials (COU-AA-301 and COU-AA-302) (De Bono et al. 2011, Ryan et al. 2013).

COU-AA-301 enrolled 1195 patients with mCRPC previously treated with docetaxel and randomized them in a 2:1 ratio to AA (1000 mg/day) with prednisone (5 mg twice daily) or to placebo plus prednisone (5 mg twice daily) (De Bono et al. 2011). At a median follow-up of 20.2 months, median OS in the AA group was 15.8 months versus 11.2 months in the placebo group (HR 0.74; 95% CI 0.64–0.86; P<0.0001) (Fizazi et al. 2012). The most common grade 3 and 4 AEs in the AA group were fatigue (9%), anemia (8%), back pain (7%), and bone pain (6%) (Fizazi et al. 2012). On the basis of the survival advantage demonstrated, AA plus prednisone was FDA approved in April 2011 for men with CRPC who progressed after docetaxel-based therapy.

COU-AA-302 randomly assigned 1088 patients with chemotherapy-naïve mCRPC to AA plus prednisone or placebo plus prednisone. After a median follow-up of 22.2 months, median OS in the placebo group was 27.2 months compared with the AA group where median OS was not reached (HR 0.75; 95% CI 0.61–0.93; P=0.01) (Ryan et al. 2013). In addition, results presented recently at the European Society for Medical Oncology 2014 annual meeting have indicated that after a median follow-up of 49.4 months, there was significantly prolonged median OS for the AA plus prednisone arm compared with the prednisone-only arm (34.7 months versus 30.3 months; HR 0.80; 95% CI 0.69–0.93; P=0.0027) (Ryan et al. 2015). In the initial analysis, the other co-primary endpoint, rPFS, also showed benefit for the AA group with a median rPFS of 16.5 months compared with 8.3 months for placebo (HR 0.53; 95% CI 0.35–0.52; P<0.001) (Ryan et al. 2013).

Key secondary endpoints also favored the AA group; these included time to initiation of cytotoxic chemotherapy, opiate use for cancer-related pain, PSA progression, and decline in performance status. The AEs associated with mineralocorticoid excess and liver function test abnormalities, seen in previous AA trials, were again displayed (De Bono et al. 2011, Ryan et al. 2013). AA received FDA approval in December 2012 for patients with chemotherapy-naïve mCRPC (National Cancer Institute 2013).

Mechanisms of resistance toward abiraterone acetate

Similarly to the situation for enzalutamide, patients with mCRPC on AA plus prednisone eventually developed resistance (De Bono et al. 2011, Ryan et al. 2013). In 31 patients treated with AA, AR-V7 was also associated with inferior PSA response rates (0% versus 68%; P=0.004), shorter time to PSA progression (median, 1.3 months versus not reached; P<0.001), clinical or rPFS (median, 2.3 months versus not reached; P<0.001), and OS (median, 10.6 months versus not reached; P=0.006) (Antonarakis et al. 2014).

Another proposed predictive biomarker for AA response in CRPC is the TMPRSS2–ERG fusion gene. The samples taken from the initial phase I/II clinical trial evaluating AA identified 15 patients who had ERG rearrangement, 12 (80%) of whom had a PSA decline of ≥90% (Attard et al. 2009). In COU-AA-302, 117 patients with evaluable samples were identified as having the ERG rearrangement. Upon ERG subtype analysis, patients with a unique ERG subtype were found to have a nonsignificant trend toward rPFS and time to PSA progression compared with the ERG nonrearrangement cohort (Attard et al. 2015). However, this biomarker remains controversial given that the results of another study did not indicate the presence of the TMPRSS2–ERG fusion on circulating tumor cells to be associated with AA response (Danila et al. 2011).

In addition, in recent case reports a paradoxical decline in PSA in patients who stopped abiraterone acetate due to disease progression has been described, similar to the AAWD phenomenon. The underlying mechanism for this effect is unknown at this time (Gauthier et al. 2012, Caffo et al. 2013).

Breast cancer

Unlike PCa, the antiandrogen narrative for BCa is in its infancy, despite knowledge of AR expression in BCa reaching back almost 50 years (Heuson et al. 1973, Engelman et al. 1974).

The AR in BCa

Historically, androgens were considered beneficial for the treatment of BCa. Before the 1970s, BCa was treated with DHT, testosterone, and fluoxymesterone with some clinical efficacy (Adair & Hermann 1946, Kennedy 1958, McNamara et al. 2014). However, androgen therapy fell out of favor due to concerns regarding aromatization to estrogen, virilizing effects, and the availability of the estrogen-targeted therapy tamoxifen (Kennedy 1958, 1959).

Results of early epidemiological studies were indicative of an association between BCa incidence and higher levels of circulating androgens (Berrino et al. 1996, Dorgan et al. 2010). Results of retrospective analyses indicate an increased risk of BCa in pre- and post-menopausal women with higher levels of estrogens, testosterone, and adrenal androgens (Berrino et al. 1996, Key et al. 2002, Page et al. 2004, Eliassen et al. 2006, Dorgan et al. 2010). Results of studies using animal models revealed that combined testosterone and estrogens induce proliferation in breast tissue, overexpression of AR, and activation of estrogen-responsive genes, all of which are reversed by anti-androgen therapy (Xie et al. 1999, Wong & Xie 2001, Nantelmet et al. 2005). In vitro, differing responses to androgen administration are observed for different BCa cell lines. Growth of MCF-7 (estrogen receptor (ER)-positive) and human epidermal growth factor receptor 2 (HER2)-negative) and MDA-MB-453 (ER – HER2 – / +) cells were promoted by the presence of androgens (DHT and nonmetabolized synthetic mibolerone), whereas growth of T47-D (ER+HER2 – ) and ZR-75-1 (ER+HER2 +) cells was inhibited by androgens. These changes were reversed by the AR antagonist, hydroxyflutamide (Birrell et al. 1995). The differing response to androgens in breast cell lines implies variable factors involved in AR signaling.

Clinically, AR is highly expressed in both normal and malignant breast tissue, with positive expression defined as immunohistochemical (IHC) nuclear staining ≥1% or ≥10%, depending on the study (Ogawa et al. 2008, He et al. 2012, Tang et al. 2012, Gucalp et al. 2013, Safarpour et al. 2014). The proportion of tumors co-expressing AR varies depending on the intrinsic subtype of BCa. For example, in ER+, HER2+, and triple-negative BCa (TNBC), prevalence of AR according to IHC is 70–95, 50–81, and 12.5–35% respectively (Agoff et al. 2003, Micello et al. 2010, Park et al. 2010, Hu et al. 2011, Cochrane et al. 2014, Safarpour et al. 2015, Choi et al. 2015). When classifying BCa by gene expression-derived intrinsic subtype, luminal A represents the highest proportion of AR+ tumors (91%) and basal-like (32%) demonstrates the lowest, with luminal B (68%) and HER2 (59%) having intermediate values (Isola 1993, Agoff et al. 2003, Collins et al. 2011, Lehmann et al. 2011, Loibl et al. 2011, Safarpour et al. 2014).

While AR is expressed across all subtypes of BCa, the prognostic role is not fully established and evidence is occasionally conflicting. The association between AR expression and survival appears to be linked to tumor subtype, nature of treatment, and stage of disease. In a study of a series of 215 women, co-expression of ER and AR conferred a survival advantage (AR + 94% versus AR− 75%; P=0.0002), but AR expression had no effect on survival in the ER− tumors (AR+ 60% versus AR− 70%; P=0.32) (Peters et al. 2009). In a separate study of a series of 347 women, improved OS was seen with AR expression independent of ER/progesterone receptor (PR) status (79% in AR+ versus 64% in AR−; P=0.004) (Gonzalez-Angulo et al. 2009). Although AR is often associated with improved clinicopathological features compared with AR-nomexpressing tumors, this is not universal and in some series AR expression was linked to poorer OS or no improvement in OS (Agoff et al. 2003, Park et al. 2010). In a cohort of 492 women with TNBC (17.7% AR+), AR expression correlated with older age (P<0.001), poor OS (P=0.008) and decreased disease-free survival (DFS) (P=0.011). In the stratified analysis, DFS and OS were driven by patients with early-stage disease not related to lymph node involvement or metastatic disease (Choi et al. 2015). In an earlier study of BCa patients treated with tamoxifen, lack of AR expression was associated with poorer response to therapy and a trend toward decreased survival compared with AR+ tumors (Bryan et al. 1984). In women treated with neoadjuvant chemotherapy, AR expression was associated with improved DFS and OS, compared with tumors not expressing AR (Loibl et al. 2011). Results of post-treatment stratified analysis demonstrated that AR expression predicted better DFS (85.7% versus 65.5%) and OS (95.2% versus 76.2%) in the TNBC group, but not in the other predefined subgroups including luminal A, luminal B, and HER2 (Loibl et al. 2011). In women with TNBC, decreased rates of pathological complete response were observed after neoadjuvant anthracycline-based chemotherapy (10% in a new TNBC subtype called ‘luminal AR’ (LAR) versus 52% in other TNBC subtypes) (Lehmann et al. 2011, Masuda et al. 2013). Conversely, there is evidence that AR expression is predictive of chemotherapy responsiveness in ER+ disease, with improved 5-year event-free survival in tumors with high AR mRNA expression (74% versus 57%; P=0.013) and shorter event-free survival when AR mRNA expression was low (odds ratio (OR) 2.86; 95% CI 1.29–6.35; P=0.01), adjusted for HER2, Ki67, tumor size, age, and tumor grade (Witzel et al. 2013).

It is likely that AR has a role across all BCa subtypes, yet its value as a prognostic marker remains unclear. In fact, the
significance of AR may vary across subtypes as a result of its varying relationship with ER in the different subtypes. We first review AR in the context of TNBC, where the first androgen-dependent BCa growth was identified and subsequently exploited as an investigational therapeutic target.

**Triple-negative BCa**

Doane et al. (2006) identified a subset of ER− breast tumors with a gene expression profile similar to that of ER+ BCa, including genes that are targets of or responsive to androgen. In a separate series of 587 TNBC tumors, six distinct subgroups were recognized based upon their molecular profiles; one of these confirmed the previously described ER−/AR+ subtype and was termed LAR (Lehmann et al. 2011). Compared with other TNBC subtypes, the LAR tumors reveal a greater than tenfold (P<0.004) protein expression of AR and are enriched for hormone-regulated-pathway genes, including genes involved in steroid synthesis, porphyrin metabolism, and androgen/estrogen metabolism. The most highly expressed genes are downstream of AR and classify a luminal gene expression profile (Doane et al. 2006, Lehmann et al. 2011). The LAR subtype demonstrated similarities to a previously identified histologically distinct subtype, molecular apocrine (Birrell et al. 1998, Farmer et al. 2005). When tumors were selected based on histologic apocrine features, gene expression profile highly correlated with the LAR subtype, indicating that the LAR TNBC group includes tumors classified with molecular apocrine histology (Lehmann et al. 2011).

Doane and colleagues went on to find a cell line, MDA-MB-453, which recapitulated the molecular profile of the LAR subtype with profile of AR+ /ER− /PR− /HER2− (+) (low), depending on the testing laboratory (Vranic et al. 2011). In preclinical experiments, these cells exhibit androgen-dependent growth, and are inhibited by the AR antagonist flutamide in an estrogen-independent manner (Farmer et al. 2005, Doane et al. 2006). These results led to the generation of new hypotheses and to the first, proof of concept trial for androgen blockade in patients selected by AR status with metastatic ER/PR− BCa.

**Bicalutamide as treatment for AR+ TNBC**

Bicalutamide treatment results in enhanced cell death in the AR+ MDA-MB-453 BCa cell line with reduction of HER3 and pAKT signaling (Ni et al. 2011). The Translational Breast Cancer Research Consortium (TBCRC) led the first, proof of concept, phase II trial testing the antitumor activity of bicalutamide at a dose of 150 mg daily in women with metastatic TNBC, selected by AR status. TBCRC011 screened over 450 patients for AR status using IHC testing of primary or metastatic tumors. Of the patients tested 12% were AR+, defined as >10% nuclear staining. This trial met its prespecified endpoint of clinical benefit rate (CBR; defined as complete response, partial response, or stable disease >24 weeks) of 19% (95% CI 5−42%) with a median PFS of 12 weeks (Gucalp et al. 2013). Bicalutamide was well tolerated with no grade 4 or 5 AEs. Grade 3 toxicities were limited to elevated liver function tests in three patients. Grade 1 events that occurred in >10% of patients (n=28) included AST/ALT elevation (n=10), hot flashes (n=6), limb edema (n=6), and fatigue (n=5) (Gucalp et al. 2013). The antitumor activity demonstrated by bicalutamide is highly compelling and similar to that seen in chemotherapy trials for the treatment of TNBC unselected by AR status.

Moreover, the benefit of an anti-AR approach in AR+ TNBC was recently described in a case report of the experience of a woman with AR 100%, ER−/PR−/HER2− metastatic BCa who had progressive disease despite six lines of cytotoxic chemotherapy. She achieved a complete response after 4 months of treatment with bicalutamide and remained disease-free for >12 months at the time of publication (Arce-Salinas et al. 2014). Although anecdotal, this is the first report, to our knowledge, of a response with the utilization of AR-targeted therapy in a carefully selected population with androgen-driven disease.

**Enzalutamide in AR+ TNBC** Building upon the promise of next-generation AR antagonists developed for the treatment of PCa, investigators have begun to study these agents in AR+ BCa. In a phase I dose-escalation trial in patients with advanced BCa, enzalutamide was found to be well tolerated at 160 mg daily with common (>10%) treatment-related AEs including nausea, vomiting, and fatigue; no treatment-related AEs ≥ grade 3 were reported (Traina et al. 2013, Schwartzberg et al. 2014). For patients with AR+ metastatic TNBC, a phase II trial of single-agent enzalutamide has completed accrual (NCT01889238) and stage 1 results were recently presented (Traina et al. 2013, 2014). IHC testing showed ≥10% AR-positivity in approximately 55% of tumors screened. The primary endpoint is CBR ≥16 weeks; secondary endpoints include CBR ≥24 weeks, PFS, response rate, and toxicity. In 26 evaluable patients, CBR at 16 weeks was 42% (11/26) and CBR at 24 weeks was 35% with one partial response and one complete response. The stage 2 data continue to mature, but statistical boundaries for efficacy have already been achieved such that the null hypothesis was rejected (Traina et al. 2014).
In addition, correlatives were an important part of this trial design, and progress has been made in defining a companion biomarker of response to enzalutamide.

**ER-positive BCa**

The AR is highly co-expressed in ER-positive cells, with expression ranging between 70 and 95% (Isola 1993, Agoff et al. 2003, Collins et al. 2011, Lehmann et al. 2011, Loibl et al. 2011, Safpour et al. 2014). Although the functional relationship of the AR and ER is not completely understood, ER-AR cross talk has been indicated by preclinical data. The domains of AR and ERα can physically interact, leading to transcriptional consequences. For example, in the presence of estradiol (E2), the N-terminus of the AR can interact with the LBD of ERα, leading to a transcriptional inhibitory interaction in both receptors (Panet-Raymond et al. 2000). Transfection of the AR DBD in BCa cells is sufficient to inhibit ERα activity, indicating a direct competition for binding sites. ARA70, an AR cofactor, also interacts with ERα and AR, and has demonstrated the ability to bind to estrogen response elements (Panet-Raymond et al. 2000, Peters et al. 2009, Fioretti et al. 2014).

Understanding the relationship between ER and AR signaling pathways may be most relevant when discussing its role in anti-estrogen therapy. In patients with previously treated ER+ disease, increased androgen production is considered a potential indicator for resistance to anti-estrogen therapy and a possible pathway for growth and survival of tumor in treated patients. Androgens are converted by aromatase to estrone and E2. Aromatase inhibitors (AIs) block the conversion of androgens to estrogens by inhibiting this enzyme, resulting in an increase in androgen levels (Cochrane et al. 2009, Peters et al. 2009, Fioretti et al. 2014). Mouse models treated with an AI demonstrate markedly elevated intratumoral testosterone concentrations (Wong & Xie 2001). In women on AI therapy, circulating testosterone, androstenedione, and DHEAS levels are increased and high levels of adrenal androgen before treatment can predict a propensity for AI failure (Morris et al. 2001). Transfection of AR into ER+ cells confers resistance to both AI therapy and tamoxifen (De Amicis et al. 2010). Exogenous overexpression of AR renders the ER+ MCF-7 BCa cell lines resistant to inhibitory effects of tamoxifen in growth assays and in xenograft models (De Amicis et al. 2010, Peters et al. 2012). Furthermore, the AR-overexpressing cells remain sensitive to growth with DHT and inhibition with bicalutamide (De Amicis et al. 2010). To further delineate this relationship, Peters and colleagues looked at the AR:ER ratio. The results indicated that a high ratio of AR:ER, rather than absolute AR expression level, may be more predictive of tamoxifen failure. Results of *in vitro* studies indicate that E2-induced proliferation of T-47D and ZR-75-1 BCa cell lines was decreased when cells demonstrated an increased AR:ER ratio (Peters et al. 2009). Overall, AR expression in ER+ cancers is associated with good prognostic indicators including improved OS and DFS, lower grade, smaller tumor size, and lack of lymph node involvement at diagnosis (Gonzalez-Angulo et al. 2009, Peters et al. 2009, Castellano et al. 2010, Niemeier et al. 2010, Tokunaga et al. 2013, Schwartzberg et al. 2014). However, AR appears to predict resistance or decreased responsiveness to anti-estrogen therapy (Bryan et al. 1984, De Amicis et al. 2010, D’Amato et al. 2013). These results make combined AR and ER targeting appealing.

**Enzalutamide in ER+ BCa** In BCa cell lines, enzalutamide abrogated androgen-mediated proliferation of ER+ (MCF-7, BCK4, T47D and ZR-75-1) and ER− BCa cell lines (MDA-MB-453). Specifically, in MCF-7 and BCK4 (AR+ER+) and MDA-MB-453 (AR+ER−) cell lines, proliferation stimulated by DHT and DHT-mediated growth was blocked by enzalutamide (Cochrane et al. 2014). Xenograft models exhibited similar results with reduction in tumor size when oral enzalutamide was administered. This occurred in a dose-dependent fashion (Cochrane et al. 2014).

The previously described phase I trial included expansion cohorts, which tested enzalutamide in combination with exemestane, anastrozole, or fulvestrant. Notably, enzalutamide induces CYP3A4, an enzyme involved in the metabolism of exemestane and anastrozole. Concurrent treatment with enzalutamide reduced exemestane exposure by approximately 40% due to this interaction. Doubling the dose, as recommended by the US product label, when combined with potent CYP3A4 inducer appears to restore the exemestane exposure (Schwartzberg et al. 2014). Anastrozole exposure was reduced by nearly 90% with concurrent enzalutamide, therefore the combination is not being further developed. Study of the cohort treated with enzalutamide and fulvestrant is ongoing (NCT01597193), but not expected to encounter similar drug interactions. A randomized, placebo-controlled, phase II trial is evaluating exemestane with or without enzalutamide in 240 patients with ER/PR+/HER2− advanced BCa (Schwartzberg et al. 2014). The co-primary endpoint is PFS in all patients and in patients with AR+ disease. Crossover is allowed following RECIST 1.1 progression. Secondary endpoints include CBR at ≥24 weeks, response rate, duration of response, safety, and tolerability.
AA in ER+ BCa  On the basis of the mechanism of action of AA as a CYP17 inhibitor, there was a rationale for studying its potential benefit in ER+ BCa, as reduced levels of E2 are expected from upstream inhibition of the steroid synthesis pathway. A large, prospective, phase II trial randomized 300 post-menopausal women with ER+ metastatic BCa to one of three treatment arms: i) exemestane, ii) exemestane with AA and prednisone, or iii) AA and prednisone. The primary endpoint of the study was PFS. Results of an interim analysis led to early closure of the AA plus prednisone arm due to futility. At the final analysis, there was no significant difference in median PFS between the combination arm and the exemestane-alone arm (HR 0.96; 95% CI 0.70–1.32; P=0.795) or for the AA plus prednisone arm compared with exemestane-alone (HR 1.1; 95% CI 0.82–1.60; P=0.437). Treatment was relatively well-tolerated, and no unexpected AEs occurred. Grade ≥3 AEs including hypokalemia, hypertension, and AST/ALT elevation were mitigated by concurrent prednisone, yet were numerically more common in the AA treatment arms (O’Shaughnessy et al. 2014).

The failure of abiraterone to significantly improve outcome in this patient population may be related to pathways already discussed. First, the importance of the GR should not be underestimated. It has been proposed that the concurrent requirement for prednisone with abiraterone may activate signaling through the GR, thereby potentiating tumor growth. Second, although abiraterone effectively reduced E2 levels in pharmacokinetic correlatives associated with this trial, progesterone levels were significantly increased above that of typical physiologic levels (O’Shaughnessy et al. 2014). Third, CYP17A1 inhibition simultaneously decreases testosterone in certain cell lines (i.e., T47-D and ZR-75-1), the androgen has demonstrated protective effects which may be lost with CYP17 inhibition (Birrell et al. 1995). The complex cross talk between AR and ER pathways may counter the benefits of reduced E2 levels. Ongoing correlative analyses are anticipated which may clarify the role, if any, for AA in the treatment of BCa.

HER2-positive BCa

AR is enriched in ER−/HER2+ BCa, with 77% of HER2-amplified tumors expressing AR, compared with 30% of HER2− tumors (Micello et al. 2010). Preclinical observations indicate that hyperactivation of HER2 enhances AR and that AR upregulation is involved in a positive feedback loop with HER2 (Ni et al. 2011). AR mediates ligand-dependent activation of WNT and HER2 signaling pathways, through direct transcription of WNT7B and HER3 and collaboration between AR and FOXA1 transcriptional activation (Ni et al. 2011). In some series, survival appears unaffected by AR status, whereas others indicate more favorable outcomes when HER2 and AR are co-expressed (Gonzalez-Angulo et al. 2009, Micello et al. 2010, Arslan et al. 2012, Lin et al. 2012). Further study regarding the role of AR in HER2+ disease is warranted (Niemeier et al. 2010). Trials for the combination of HER2 blockade with androgen blockade using enzalutamide and trastuzumab are ongoing (NCT02091960).

Novel agents

Despite hormone deprivation therapy, tumors eventually progress to a castration-resistant state in PCa through AR overexpression or gene amplification, increased intratumoral androgen synthesis, aberrant AR expression (either by mutation or splice variants), and activation of alternative pathways (Ford et al. 2003, Chen et al. 2004, Stanbrough et al. 2006, Montgomery et al. 2008). Resistance to anti-estrogen therapy similarly occurs for patients with ER-driven advanced BCa and likewise for AR-driven BCa. An increased recognition of the mechanism utilized by the AR in a castrate state has led to the development and subsequent approval of enzalutamide and AA, both shown to improve OS for men with mCRPC (Fizazi et al. 2012, Scher et al. 2012, Beer et al. 2014). Investigations of women with BCa are ongoing but offer hope for a potential, highly effective strategy for endocrine manipulation.

Novel therapies in various stages of development inhibiting aspects of the AR pathway include LBD inhibitors (ARN-509, ODM-201), androgen synthesis inhibitors (TOK-001, TAK-700, VT-464), selective AR downregulators (AZD-3514), selective AR modulators (GTx-024), and target chaperone inhibitors (OGX-427, AT13387, STA-9090). These are described below and in Tables 1–3 and illustrated in Fig. 2.

Next-generation AR antagonists: targeting the LBD

ARN-509 is a second-generation AR antagonist with a mechanism of action similar to that of enzalutamide in that after binding to the LBD of the AR, it inhibits AR nuclear translocation and AR binding to androgen response elements. However, ARN-509 preclinical data have indicated lower steady-state brain levels than those obtained with enzalutamide, indicating a lower seizure potential with ARN-509 (Clegg et al. 2012, Scher et al. 2012).
Phase I data indicate a linear pharmacokinetic profile and potential efficacy of the drug with a PSA decline defined as \( R \geq 50\% \) from baseline in 46.7% of patients at 12 weeks. Treatment was well tolerated in the phase I trial, with the most frequent AEs reported being grade 1/2 fatigue in 47% of patients. Notably, no seizure activity occurred at any dosing level in the phase I study (Rathkopf et al. 2013a,b).

A phase II clinical trial of ARN-509 is currently ongoing on three cohorts of patients with CRPC, including i) treatment-naïve high-risk non-metastatic CRPC, ii) treatment-naïve mCRPC, and iii) AA-exposed mCRPC. Preliminary results indicated a PSA response rate (based on the PCa Working Group 2 criteria) at 12 weeks of 91% for the high-risk treatment-naïve non-metastatic CRPC arm, 88% for the treatment-naïve mCRPC arm, and 29% for the AA-exposed mCRPC population (Rathkopf et al. 2013a, Smith et al. 2013). ARN-509 is now being studied in a phase III randomized placebo-controlled clinical trial in men with non-metastatic CRPC (SPARTAN; NCT01946204) as well as in a phase I clinical trial in combination with AA in men with mCRPC (NCT02123758). ARN-509 preclinical data also indicated antitumor effects in the BCa cell line MDA-MB-453 cells (Clegg et al. 2012) though the study has not advanced past the preclinical stage.

ODM-201 is a next-generation AR inhibitor which demonstrates binding of WT AR with higher affinity than enzalutamide, blocks nuclear translocation, and does not demonstrate agonist activity when AR is overexpressed nor experience CNS penetration, thus decreasing or eliminating its potential seizure activity (Moilanen et al. 2013).

The phase I/II data from the ARADES trial supports its use as monotherapy in men with mCRPC, showing a tolerable safety profile. The most common AEs in included fatigue (12%), hot flashes (5%), and decreased appetite (4%). One grade 3 AE was reported, fatigue, with no grade 4 AEs. PSA response at
12 weeks (defined as ≥50% decrease from baseline) was obtained in 13 out of 15 (87%) patients. All patients achieved partial response or stable disease by RECIST v1.1 criteria (Fizazi et al. 2013). The phase II data revealed a PSA response at 12 weeks in 29, 33, and 33% of the cohorts receiving doses of 200, 400, and 1400 mg respectively (Fizazi et al. 2014).

Currently, a phase III randomized placebo-controlled clinical trial is ongoing in patients with high-risk non-metastatic CRPC, the ARAMIS trial (NCT02200614).

### Novel androgen synthesis inhibitors

In contrast to targeting the LBD of the AR, inhibition of androgen synthesis with novel agents remains another viable strategy. TOK-001 (galetorone) is a semi-synthetic steroid analog which inhibits PCa cell growth by functioning as a CYP17 lyase inhibitor, and an AR antagonist for both WT and mutant ARs as well as degrading AR protein. Results of the phase I trial, ARMOR1, showed PSA reduction ≥30% in 24 patients (49%), including 11 (22%) who had ≥50% reduction in PSA (Taplin et al. 2012). The phase II trial ARMOR2 (NCT01709734) is ongoing (Taplin et al. 2014). Preliminary data from the phase II trial indicated that the drug was well tolerated, with most AEs (94%) being grade 1 or 2 and including nausea, diarrhea, fatigue, and pruritus.

TAK-700 (oritronel) is a nonsteroidal oral androgen synthesis inhibitor, with affinity for 17α-hydroxylase. Development of TAK-700 in PCa has been halted given that two large phase III trials in chemotherapy-naïve (ELM-PC 4) and chemotherapy-exposed (ELM-PC 5) patients with mCRPC, both had negative results and showed no OS benefit (Takeda Pharmaceutical Company Limited 2014, De Wit et al. 2014, Fizazi et al. 2015). A potential hypothesis explaining these negative findings is that TAK-700 was tested in a patient population exposed to a number of currently available agents, including AA, that could have influenced the phase III results.

TAK-700 is currently under investigation in metastatic BCa (NCT01808040, NCT01990209). Preclinical activity of TAK-700 provided the basis of this investigation; TAK-700 does not directly inhibit aromatase activity, but at a dose of 300 mg/kg it appears to suppress serum E2, testosterone, androstenedione, and 17-hydroxyprogesterone to a similar degree as 0.1 mg/kg of anastrozole. In animal models, TAK-700 appears to suppress serum E2 concentrations in hypophysectomized female rats and monkeys through the inhibition of CYP17A1 activity.

### Table 3 Novel agents targeting the AR axis in late-stage development for breast cancer

<table>
<thead>
<tr>
<th>Agent</th>
<th>Phase</th>
<th>Population</th>
<th>Notes</th>
<th>References/clinicaltrials.gov</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AR antagonist</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicalutamide</td>
<td>II</td>
<td>AR + mTNBC</td>
<td>Gucaip et al. (2013)</td>
<td>NCT00468715 (closed)</td>
</tr>
<tr>
<td>Enzalutamide (MDV3100)</td>
<td>II</td>
<td>AR + mTNBC</td>
<td></td>
<td>NCT01889238 (active, not recruiting)</td>
</tr>
<tr>
<td><strong>Biosynthesis inhibitor</strong></td>
<td></td>
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</tr>
<tr>
<td>Abiraterone acetate</td>
<td>I/II</td>
<td>ER+ or AR+ mBCa, post-menopausal</td>
<td></td>
<td>NCT00755885 (active, not recruiting)</td>
</tr>
<tr>
<td>Abiraterone acetate</td>
<td>II</td>
<td>AR + TNBC, molecular apocrine</td>
<td></td>
<td>NCT01842321 (recruiting)</td>
</tr>
<tr>
<td>Orteronel (TAK-700)</td>
<td>Ib</td>
<td>HR + mBCa</td>
<td>Prostate (Dreicer et al. (2014))</td>
<td>NCT01808040 (suspended)</td>
</tr>
<tr>
<td><strong>Orteronel (TAK-700)</strong></td>
<td>II</td>
<td>HR + mBCa</td>
<td></td>
<td>NCT01990209 (recruiting)</td>
</tr>
<tr>
<td>Targeting the AR carrier molecule</td>
<td>II</td>
<td>mBCa</td>
<td>Small-molecule HSP90 inhibitor</td>
<td>NCT01273896 (completed results pending)</td>
</tr>
<tr>
<td>Ganetespib (STA-9090)</td>
<td>II</td>
<td>mBCa (TNBC, ER +, HER2 +)</td>
<td>Small-molecule HSP90 inhibitor</td>
<td>NCT01677455 (active, not recruiting)</td>
</tr>
<tr>
<td><strong>Selective AR modulators (SARMs)</strong></td>
<td></td>
<td>mBCa</td>
<td></td>
<td></td>
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<tr>
<td>Enobosarm (GTx-024)</td>
<td>II</td>
<td>mBCa</td>
<td></td>
<td>NCT01616758 (active, not recruiting)</td>
</tr>
<tr>
<td><strong>Combination trials</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Trastuzumab + enzalutamide</td>
<td>II</td>
<td>mBCa, AR +, HER2 +</td>
<td></td>
<td>NCT02091960 (recruiting)</td>
</tr>
<tr>
<td>Fulvestrant + enzalutamide</td>
<td>I</td>
<td>ER + AR + mBCa</td>
<td></td>
<td>NCT01597193 (active, not recruiting)</td>
</tr>
<tr>
<td>Exemestane + abiraterone</td>
<td>II</td>
<td>mBCa, ER +</td>
<td>O’Shaughnessy et al. (2014)</td>
<td>NCT01381874 (active, not recruiting)</td>
</tr>
</tbody>
</table>

AR, androgen receptor; mTNBC, metastatic triple-negative breast cancer; ER, estrogen receptor; mBCa, metastatic breast cancer; TNBC, triple-negative breast cancer; HR, hormone receptor; HER2, human EGF receptor 2; HSP, heat shock protein.
indicating a possible role for this agent in hormone-dependent BCa (Yamaoka et al. 2013).

VT-464, currently under investigation for PCa, is an oral nonsteroidal selective inhibitor of CYP17,20 lyase with less activity toward CYP17 hydroxylase, therefore eliminating the need for concurrent steroid administration (Eisner et al. 2012, Figg et al. 2012). Preclinical results have indicated that cell lines treated with VT-464 had significantly lower levels of testosterone and DHT compared with abiraterone-treated cells, indicating a greater effect on AR pathway suppression with VT-464 (Toren et al. 2015). Both a phase I and a phase II clinical trial are actively recruiting patients (NCT02012920, NCT02130700).

Selective AR modulators

Selective AR modulators (SARMs) are a class of drugs in development; unlike androgen synthesis inhibitors, they act as selective androgen agonists and show promise as a potential therapeutic strategy in BCa. Enobosarm (GTx-024) is the farthest along in clinical development, and demonstrates an agonist effect in some populations inhibits BCa growth. Preclinical data show antitumor activity of GTx-024 in AR+ stably expressing cell lines MCF-7 (ER+) and MDA-MB-231 (TNBC) implanted subcutaneously into nude mice. Tumor growth was reduced more than 75% in MDA-MB-231-AR cells and 50% in MCF-7-AR cells compared with vehicle-treated tumors, demonstrating benefit (Dalton et al. 2013). However, it is uncertain how to best select the patients who may benefit from this therapeutic strategy. A phase II trial is open to accrual for the treatment of women with ER+ metastatic BCa (NCT01616758). The primary endpoint is clinical benefit at 6 months as measured by RECIST, with secondary objectives including objective response rate, PFS, and response in the AR+ subset.

Chaperone inhibitors

As shown in Fig. 2, targeting the AR chaperone is another rational strategy currently under development. Heat shock proteins (HSP) are stress proteins functioning as molecular chaperones that play a variety of roles in hormone signaling pathways and transcription. They are transcriptionally upregulated in response to heat and stress, helping
to protect cells from damage (Taipale et al. 2010, Hong et al. 2013). In AR signaling, molecular chaperones such as HSP90 assist in protein folding, trafficking, activation, and transcription of AR. HSP90 maintains AR in high-affinity ligand-binding conformation to facilitate efficient response to its ligand (DHT). Clinical trials evaluating HSP90 inhibitors AT13387 and STA-9090 and an HSP27 inhibitor, OGX-427, are under development for both PCa (Table 2) and BCa (Table 3).

Preclinical activity of ganetespib (STA-9090) demonstrated decreased viability of PCa cell lines, independent of androgen sensitivity or AR status (He et al. 2013). However, the phase II trial of single-agent ganetespib in heavily pretreated men with CRPC failed to reach its primary endpoint of 6-month PFS. The trial was halted early due to 6-month PFS of 1.9 months (90% CI 1.7–2.7 months) with median OS of 10.2 months (90% CI 2.3–18.3 months). The compound is no longer in development for PCa (Heath et al. 2013). In BCa, ganetespib preclinical data indicate activity in hormone-receptor-positive (MCF-7, T47D), HER2-overexpressing (BT-474, Sk-BR3), TNBC (MDA-MB-231, OCUB-M) and inflammatory (SUM 149) BCa cell lines, with the HER2+ cell line BT-474 being the most sensitive. Treatment of hormone-receptor-positive cell lines led to potent dose-dependent destabilization of ER and PR, accompanied by increased HSP70 expression, a surrogate marker for HSP90 inhibition. In HER2+ cell lines, activated HER2 was degraded after exposure to ganetespib. In MCF-7 and MDA-MB-231 xenografts, ganetespib suppressed tumor growth and led to tumor regression in a BT-474 model (Friedland et al. 2014). Clinical trials are ongoing for BCa; however, these have been primarily focused on patients with HER2+ tumors on the basis of the preclinical data (NCT01273896; NCT02060253).

**Dual inhibition of AR and other signaling pathways**

In understanding the AR pathway, additional signaling pathways have been identified with potential implications in mediating AR downstream activity. Pathways include PI3K/AKT/MAPK, PTEN, p53, and cell-cycle regulators. Several novel therapies are in development on the basis of the hypothesis that dual inhibition of these companion pathways will be superior to single-agent anti-androgen therapy.

**Enzalutamide and abiraterone acetate** Dual AR pathway inhibition is currently being evaluated by combining enzalutamide and AA in men with mCRPC. Interim results of an early-phase clinical trial indicated this combination approach to be well tolerated, with grade 3 AEs being elevated ALT, hypertension, and increases in alkaline phosphatase, arthralgias, and bone pain. PSA decline (defined as ≥50% decline from baseline) was seen in 76% (37/49) of patients, of which 22/49 (45%) had a ≥90% decline in PSA (Efstathiou et al. 2014). A phase III randomized trial of enzalutamide versus enzalutamide plus AA and prednisone in men with mCRPC is currently ongoing (NCT01949337).

**PI3K/AKT/mTOR** There is much attention given to alternative pathway activation in CRPC, particularly of the PI3K/AKT/mTOR pathway. Early-phase clinical trials in CRPC are ongoing to simultaneously target the AR and PI3K/AKT/mTOR pathways. For example, an ongoing phase I trial in men with progressive mCRPC following treatment with AA (NCT02106507) is evaluating the safety, PK properties, and maximum tolerated dose of ARN-509 and everolimus, an FDA-approved mTOR inhibitor used in other malignancies. In addition, abiraterone with a pan-PI3K inhibitor (BKM120) and a pan-AKT inhibitor (GDC-0068) are being investigated in mCRPC (NCT01634061, NCT01741753, and NCT01485861).

In BCa, this pathway has been a particular focus within the AR+ TNBC population, as it is enriched for PIK3CA-activating mutations (10/25, 40%; P < 0.002) compared with AR– TNBC (1/25, 4%). AR+ TNBC cell lines stimulated by DHT displayed increased PI3K pathway activation. When treated with pan-PI3K inhibitors (GDC-0941, NVP-BKM120) and dual PI3K/mTOR inhibitors (GDC-0980, NVP-BEZ235), the TNBC cell lines expressing PIK3CA mutations showed the greatest sensitivity. An additive inhibitory effect was observed in cell lines that were more sensitive to single-agent bicalutamide, including MDA-MB-453, CAL148, and SUM185 cells, when an AR antagonist (bicalutamide) was used in combination with PI3K inhibitors (GDC-0941, GDC-0980) (Lehmann & Pietenpol 2014). Clinical trials are in development.

**Conclusion**

Androgen signaling has been the primary target for the treatment of PCa. Many novel therapies are in development to overcome the mechanisms that invariably create resistance to our currently available AR antagonists and strategies for androgen deprivation. Exploiting next-generation methods for inhibiting androgen signaling offers hope for improved outcomes in patients with advanced PCa. For patients with BCa, the role of the AR and its pathway effectors is less well understood. However, we have shown for the first time, to our knowledge, that
androgen inhibition has clinical benefit for women with AR-driven ER/PR-negative BCa, thus it may offer an additional target to exploit in BCa. However, the relative importance of AR may depend upon the molecular subtype of BCa. Nevertheless, antiandrogen therapies for both PCa and BCa are exciting areas with many novel drugs in development.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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