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REVIEW PAPER

Targeting persistent androgen receptor signaling in castration-resistant prostate cancer

Laura Graham[1] · **Michael T. Schweizer**[2]

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Abstract Castration-resistant prostate cancer (CRPC), the invariably lethal phenotype of advanced prostate cancer, represents a clinical state defined by disease progression despite reduction of testosterone to castrate levels (i.e., ≤50 ng/dL). Although resistant to androgen-deprivation therapy (i.e., LHRH agonists/antagonists), CRPC continues to depend on the androgen receptor (AR)-signaling pathway. Supporting the importance of AR-signaling in a castration-resistant state, the next-generation AR-signaling inhibitors enzalutamide and abiraterone have been shown to afford a survival benefit in men with metastatic CRPC. However, primary and secondary resistance mechanisms to these agents inevitably drive continued disease progression—often as a result of re-activation of AR-signaling. With increased understanding of the mechanisms underlying how continued AR-signaling occurs in spite of drugs like abiraterone and enzalutamide, a new wave of therapies is emerging designed to more effectively target AR-signaling. This review will focus on the more clinically relevant mechanisms of CRPC drug resistance and our ongoing efforts to develop drugs to target these mechanisms.

Keywords Castration-resistant prostate cancer · Androgen receptor · Androgen receptor splice variant · Hormonal therapy · Androgen-deprivation therapy · Enzalutamide · Abiraterone · Drug resistance

✉ Michael T. Schweizer
schweize@uw.edu

[1] Department of Medicine, University of Washington, Seattle, WA 98104, USA

[2] Division of Oncology, Department of Medicine, University of Washington/Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA

Introduction

With 26,120 deaths expected in 2016, prostate cancer continues to be the second leading cause of cancer death among men [1]. Prostate cancer has been recognized as an androgen-dependent malignancy for over 75 years, and inhibiting androgen receptor (AR)-signaling through androgen-deprivation therapy (ADT) represents the first example of a targeted therapy [2]. When it was first conceived, ADT was accomplished via surgical castration; however, it is now more commonly achieved with luteinizing hormone-releasing hormone (LHRH) analogues (agonists/antagonists), which are able to reduce serum testosterone to levels equivalent to surgical castration (i.e., ≤50 ng/dL) [3, 4]. However, advanced prostate cancer inevitably progresses despite depletion of serum testosterone to castrate levels. The clinical state defined by progression of the disease while on ADT is termed castration-resistant prostate cancer (CRPC) and is ultimately fatal, with death usually occurring within 1–4 years of onset [5, 6].

It is now known that, even in this castration-resistant state, tumor growth is still reliant on AR-signaling—often as a result of persistent androgen–AR interactions. The AR is a 110-kDa protein containing four main functional domains (Fig. 1). The N-terminal domain (NTD) interacts with a number of co-regulators of AR transcription; the DNA-binding domain binds to promoter regions of AR target genes (i.e., androgen response elements, ARE); the hinge region is involved in the nuclear transport of the AR, likely through interacting with microtubules; and the

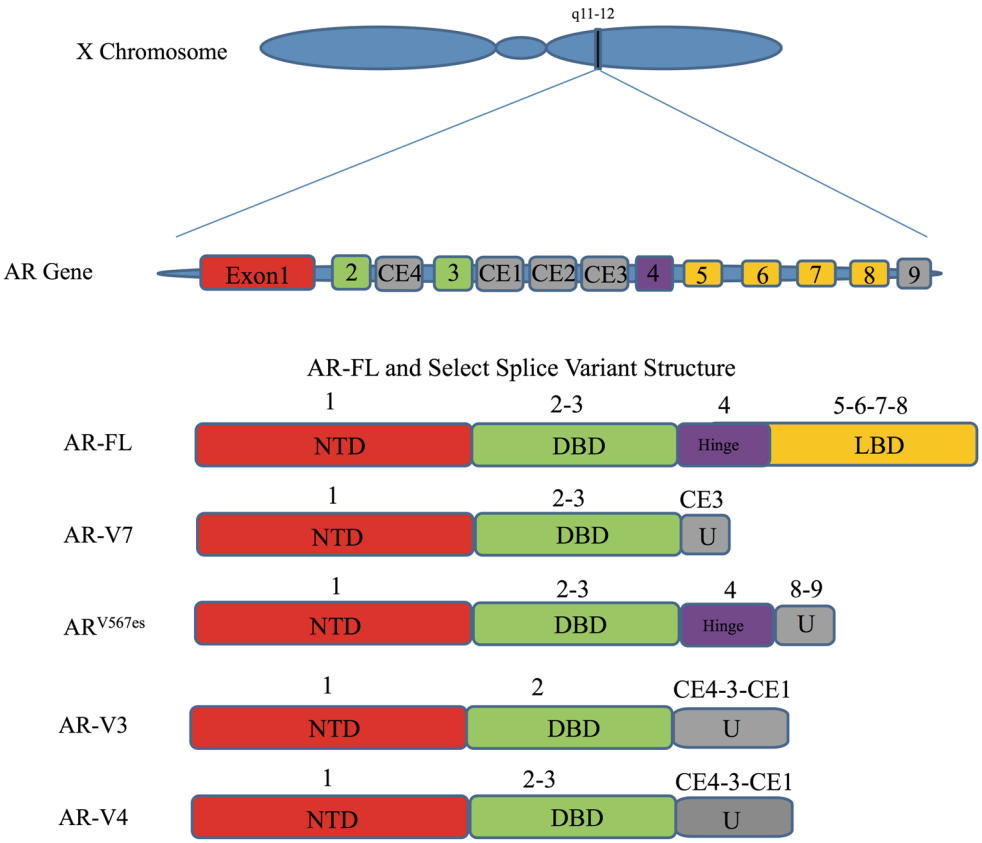

Fig. 1 The androgen receptor gene and protein with key splice variants. The androgen receptor (AR) gene is located on the X chromosome and is comprised of eight exons that code for its four distinct domains: N-terminal domain (NTD), DNA-binding domain (DBD), hinge region and ligand-binding domain (LBD). RNA can be spliced in a variety of ways and can include exons 1–8 (i.e., full-length androgen receptor, AR-FL) as well as cryptic exons (i.e., CE1-4) and exon 9. The inclusion of cryptic exons/exon 9 can result in unique (U) sequences not found in AR-FL. The structure of the four AR-splice variants (AR-Vs) known to possess constitutive activity (i.e., AR-V7, AR-v567es, AR-V3 and AR-V4) is provided along with the exons encoding the variant receptors

ligand-binding domain (LBD) binds androgens [7]. The primary ligands for the AR are androgenic steroid hormones, including testosterone and dihydrotestosterone (DHT). Upon binding to these ligands in the cytoplasm, the AR disassociates from chaperone proteins, translocates into the nucleus and subsequently undergoes homodimerization prior to binding to AREs in regulatory regions of androgen-dependent target genes, including *KLK3* which encodes for prostate-specific antigen (PSA) [8, 9]. Other targets of the AR include genes involved in prostate cell growth, angiogenesis and apoptosis—providing a mechanistic basis for the antitumor effects of ADT [10].

Serum androgens are in large part produced by the testes; however, extragonadal sources of androgens (i.e., adrenal, intraprostatic and intratumoral) are clinically important sources of androgen which are likely sufficient to fuel continued CRPC growth even when production by the testes is suppressed [11–13]. Other mechanisms likely involved in maintaining AR-signaling in a castration-resistant state include: AR overexpression, AR point mutations within the ligand-binding domain, emergence of constitutively active AR-splice variants and alternative signaling pathways able to active the AR transcriptional program [14–19].

This article provides an overview of several clinically relevant mechanisms by which AR-signaling remains engaged in men with CRPC and reviews the currently available agents targeting the ligand-binding domain of the full-length AR (AR-FL). We also discuss mechanisms of resistance to these agents and the ongoing work to develop drugs that are effective in spite of these resistance pathways.

Treatment of CRPC

First-generation antiandrogens and taxanes

In 2004 the landmark TAK-327 and SWOG-9916 trials reported that docetaxel led to an overall survival advantage compared to mitoxantrone [20, 21]. Prior to that there were no agents shown to confer a survival benefit for men with CRPC. Historically, the first-generation antiandrogens (e.g., bicalutamide, flutamide and nilutamide) had been used as frontline treatment for CRPC. These agents work by competitively inhibiting androgens from binding the LBD of the AR; however, the benefit of these agents is short lived, with progression typically occurring within 6 months [22]. Even as frontline therapy for hormone-sensitive prostate cancer, the first-generation antiandrogens by themselves are inferior to castration alone and provide only minimal benefit when combined with androgen

suppression—highlighting the need for more effective agents [23, 24]. Studies exploring the mechanisms driving resistance to the first-generation antiandrogens revealed that overexpression of the AR and the emergence of AR point mutations within the LBD are likely key mediators of disease progression [14, 19, 25]. Interestingly, these molecular events have been found to associate with the antiandrogens' ability to function as AR agonists and may underlie the antiandrogen withdrawal syndrome whereby transient disease control occurs following the cessation of antiandrogen therapy [14, 18, 19, 25, 26].

Mitoxantrone, which was compared to docetaxel in the 2004 studies, is an anthracenedione. It was approved for the treatment of CRPC on the basis of randomized studies showing that it led to improved quality of life and pain scores in men with symptomatic, metastatic CRPC (mCRPC) [27–29]. A number of additional cytotoxic chemotherapy agents were tested in men with CRPC, but it was not until the development of docetaxel, a microtubule inhibitor, that cytotoxic therapy was shown to provide a survival benefit in this patient population.

The TAX-327 trial studied treatment with prednisone and either high-dose docetaxel (75 mg/m^2) every 3 weeks, low-dose docetaxel (30 mg/m^2) given weekly or mitoxantrone in men with mCRPC. Median survival was 19.2 months in the high-dose docetaxel cohort, 17.8 months in the weekly low-dose docetaxel cohort and 16.3 months in the mitoxantrone arm. When compared to mitoxantrone, only high-dose docetaxel had a statistically significant improvement in overall survival ($P = 0.009$). The high-dose docetaxel group also had statistically significant improvements in PSA response, quality of life and pain scores. In the SWOG-9916 trial, men with CRPC were randomized to either docetaxel and estramustine or mitoxantrone and prednisone. The docetaxel and estramustine arm had statistically significant survival benefit compared with the mitoxantrone/prednisone arm (median OS 18 vs. 16 months, $P = 0.01$) [21]. Given the similar results between the TAX-327 and SWOG-9961 trials, and the fact that estramustine may result in increased toxicity, docetaxel plus prednisone has been accepted as the front-line cytotoxic regimen of choice for the treatment of mCRPC.

In the TROPIC trial, published in 2010, men with mCRPC who had progressed after docetaxel treatment were randomized to treatment with prednisone and either mitoxantrone or cabazitaxel. The median survival was 15.1 months in the cabazitaxel group and 12.7 months in the mitoxantrone group with a hazard ratio for death of 0.70 ($P < 0.0001$) [30]. This led to the approval of cabazitaxel as a second-line therapy to docetaxel. Of note, studies testing cabazitaxel in docetaxel-naïve patients are ongoing (NCT01308567, NCT01718353).

Second-generation AR-signaling inhibitors

Since 2010, the number of approved, life-prolonging therapies for the treatment of mCRPC has exploded. The observation that the AR is frequently overexpressed and androgen-regulated genes (e.g., *PSA*) are expressed in prostate cancer cells in a castration-resistant state led to a renewed interest in targeting AR-signaling in men with CRPC. The ongoing reliance of prostate cancer cells on AR-signaling is suggested in the clinical setting by the survival benefit gained with two recently approved drugs targeting the AR-signaling pathway: enzalutamide and abiraterone [31–34].

Prior to the development of the next-generation AR-directed therapies (e.g., abiraterone and enzalutamide), ketoconazole, an antifungal drug that inhibits the steroidogenic enzyme CYP-17, had been shown to lead to a reduction in androgen levels beyond those observed with the LHRH analogues alone. In a Phase III randomized trial comparing ketoconazole to an antiandrogen, ketoconazole was associated with increased PSA suppression and objective responses. Ketoconazole's use is limited, however, due to high rates of hepatotoxicity [35, 36]. Abiraterone is a newer CYP-17 inhibitor designed to inhibit extragonadal testosterone synthesis. In an open-label observational study of 57 patients with mCRPC, abiraterone treatment resulted in sustained suppression of circulating testosterone as well as testosterone in tumor-infiltrated bone marrow aspirates to an undetectable level [37].

Based on encouraging results from early-phase studies, two randomized, double-blinded, placebo-controlled Phase III trials testing abiraterone were conducted. COU-AA-301 demonstrated that in patients with mCRPC who had previously received chemotherapy, abiraterone plus prednisone prolonged overall survival by 4 months (median OS 14.8 vs. 10.9 months, $P < 0.001$), decreased time to PSA progression and improved progression-free survival compared to prednisone alone [34]. COU-AA-302 enrolled 1088 patients with mCRPC who had not previously received cytotoxic chemotherapy and found that median radiographic progression-free survival and overall survival were improved in those receiving abiraterone plus prednisone versus prednisone plus placebo (median overall survival 34.7 vs. 30.3 months, $P = 0.0033$). Importantly, patients receiving abiraterone plus prednisone as compared to prednisone alone had a delay in initiation of opiate analgesia, treatment with cytotoxic therapy and decline in performance status. Abiraterone plus prednisone also delayed PSA progression, pain onset and decline in health-related quality of life [31, 38]. Ultimately the COU-AA-301 and COU-AA-302 trials led to the FDA approval of abiraterone plus prednisone as a treatment option for mCRPC patients pre- and post-chemotherapy.

Enzalutamide is a second-generation AR antagonist that is mechanistically distinct from abiraterone. It competitively binds to the LBD of the AR, inhibits AR translocation to the cell nucleus and inhibits AR binding to DNA [39]. It has higher affinity for the AR than bicalutamide and has minimal to no agonist activity [39]. In the AFFIRM trial published in 2012, a Phase III double-blind, placebo-controlled trial, 1199 men with mCRPC who had previously received chemotherapy were randomized to receive either oral enzalutamide or placebo. Enzalutamide significantly prolonged overall survival (median OS 18.4 vs. 13.6 months, $P < 0.001$), radiographic progression-free survival and the time to first skeletal-related event [33]. In the PREVAIL trial, published in 2014, 1717 patients with mCRPC who had not received previous cytotoxic chemotherapy were randomized to receive either enzalutamide or placebo once daily. Enzalutamide treatment extended radiographic progression-free survival and overall survival (median OS 32.4 vs. 30.2 months, $P < 0.001$) and delayed the initiation of chemotherapy by a median of 17 months [32]. Based on these trials, the FDA approved enzalutamide in both the pre- and post-chemotherapy space.

While abiraterone and enzalutamide have been accepted as standard treatment options for men with mCRPC, key questions remain regarding how best to incorporate them into our current treatment paradigm. For one, it is not clear whether older antiandrogens should be abandoned in favor of one of the next-generation AR-directed agents upon transition from a hormone-sensitive to castration-resistant state. The STRIVE trial was a multicenter, randomized trial of enzalutamide versus bicalutamide in men with non-metastatic ($N = 121$) or metastatic CRPC ($N = 275$). In this trial, enzalutamide was shown to significantly improve progression-free survival compared to bicalutamide in both groups [22]. These results are perhaps not surprising given enzalutamide's clear benefit for men with mCRPC. Ultimately the STRIVE trial does not address the more clinically important question of whether one treatment strategy (i.e., bicalutamide followed by enzalutamide vs. immediate treatment with enzalutamide) is superior in terms of overall survival.

Another issue that remains unresolved surrounds the issue of how to best sequence the use of the next-generation AR-directed therapies. Perhaps not surprisingly, given their similar mechanisms of action, available data indicate that there is limited benefit to using enzalutamide or abiraterone sequentially after failure on one agent. Retrospective series suggest that response rates to abiraterone are low after progression on enzalutamide, and the same is true of response rates to enzalutamide after abiraterone failure [40–42]. A prospective study examining whether one therapeutic sequence (i.e., abiraterone followed by enzalutamide vs. enzalutamide followed by abiraterone) results in superior PSA response rates is ongoing (NCT02125357). Another strategy that is actively being investigated is the combination of abiraterone and enzalutamide in the treatment of mCRPC (NCT01650194). It is worth noting that taxanes appear to retain antitumor activity after treatment with abiraterone and enzalutamide, and docetaxel remains an important therapeutic option for this patient population. However, docetaxel's activity may also be attenuated when used post-abiraterone, possibly due to cross-resistance between AR-directed therapies and docetaxel given that taxanes may inhibit AR-signaling through inhibiting microtubule-mediated AR nuclear transport [43–47].

Beyond AR-signaling inhibition

Recent work has validated non-AR therapeutic targets in mCRPC, with an immunotherapeutic and radiopharmaceutical and next-generation taxane all shown to associate with improved overall survival when used to treat mCRPC. As mentioned above, the next-generation taxane, cabazitaxel, was approved for use in men with mCRPC. Of note, this agent may retain activity in docetaxel-resistant patients due to its retained activity in spite of high *p*-glycoprotein multidrug efflux pump activity [48]. Sipuleucel-T is an autologous antigen-presenting cell-based immunotherapy in which autologous peripheral blood monocytes (PBMCs) are activated ex vivo with a recombinant fusion protein consisting of prostatic acid phosphatase fused to granulocyte–macrophage colony-stimulating factor (GM-CSF). It was shown in the IMPACT study to confer a 4.1-month survival benefit (25.8 vs. 21.7 months, $P = 0.03$) in men with asymptomatic mCRPC compared to placebo [49]. Interestingly, in spite of its survival benefit, sipuleucel-T has not been shown to decrease PSA or lead to objective tumor responses. Finally, radium-223 is a novel alpha-emitting calcium mimetic shown to prolong survival in men with bone metastatic CRPC (median OS 14 vs. 11.2 months, $P = 0.002$) and to delay skeletal-related events (e.g., pathologic fractures) [50].

Resistance to AR-signaling inhibition

Although the next-generation AR-directed therapies enzalutamide and abiraterone represent important advancements in prostate cancer therapy, their benefit is often short lived and resistance invariably occurs [16, 31–34]. A plethora of resistance mechanisms to abiraterone and enzalutamide has been described, including: (1) increased production of intratumoral androgens through overexpression of steroidogenic enzymes (e.g., CYP17A1, AKR1C3);

(2) increased androgen transport within the tumor microenvironment; (3) signaling by other nuclear hormone receptors (e.g., glucocorticoid receptor); (4) feedback pathways leading to activation of the AR transcriptional program; and (5) adaptive changes of the AR itself (e.g., upregulation, mutations, alternative splicing) [11, 12, 14, 17, 51–67]. While an in-depth review of all the relevant resistance pathways is beyond the scope of this review, we did want to briefly touch upon the more clinically relevant (i.e., druggable) pathways.

The maintenance of persistent AR-signaling is essential to CRPC cell growth. One of the key means by which this is accomplished is through aberrations in the AR pathway itself (e.g., *AR* point mutations, AR overexpression and alternative splicing), and AR pathway alterations are present in over 70 % of mCRPC cases [17]. A number of additional oncogenic signaling pathway alterations are also able to promote persistent AR transcriptional activity. Of these, the most well recognized is PI3K/Akt/mTOR signaling—a pathway linked with prostate cancer growth, migration and angiogenesis—which is upregulated in nearly 50 % of CRPC cases [17, 68, 69]. Other key pathways driving CRPC growth include: epidermal growth factor pathways, insulin-like growth factor pathways, the JAK/STAT pathway and the WNT pathway [70]. To date, efforts to target these pathways have met with varying success.

Intratumoral upregulation of androgens, even at low levels, are likely sufficient to drive continued expression of the AR transcriptional program and represent a key resistance mechanism driving CRPC growth. Supporting this is the observation that testosterone levels within the metastatic microenvironment from men with CRPC are often significantly higher than levels within primary prostate cancers from untreated men. In these metastatic tumors, the expression of genes encoding steroidogenic enzymes, including 3βHSD, AKR1C3, SRD5A2, CYP17A1 and CYP19A1, is significantly upregulated—providing a rational basis for targeting these steroidogenic enzymes [12]. Abiraterone, a CYP17 inhibitor, was largely developed to impair intratumoral androgen production. Given the complexity of the androgen biosynthesis pathway, however, the possibility remains that other steroidogenic enzymes may be able to compensate for complete CYP17 inhibition—leading to restored intratumoral androgen biosynthesis and resistance to CYP17 inhibitors.

A recent report by Taplin and colleagues found that 24 weeks of neoadjuvant LHRH agonist plus abiraterone was associated with residual prostatic adenocarcinoma in 90 % of prostatectomy specimens, with 24 % of these men also demonstrating nodal metastases at the time of prostatectomy. This study indicates that even at an early stage, prostate cancers are able to resist potent combinatorial AR-directed therapy [71]. In this case, there is indirect evidence that persistent intratumoral steroidogenesis may be one of the key mechanisms driving drug resistance. While treatment with abiraterone and a LHRH agonist was shown to significantly decrease the levels of circulating and intraprostatic androgens, a significant amount of residual DHEA-S (an androgen substrate) remained in circulation [71, 72]. In theory, this persistent serum DHEA-S could serve as a depot for intratumoral conversion to testosterone and DHT, with the final steps of conversion to these more potent androgens catalyzed by AKR1C3 [73]. Supporting this hypothesis, cell culture and xenografts models of CRPC have implicated AKR1C3 in the emergence of resistance to both enzalutamide and abiraterone, providing a plausible case for persistent intratumoral androgen biosynthesis as a driver of resistance to CYP17 inhibition [52, 74].

Another mechanism by which prostate cancer tumors may be able to maintain sufficient intratumoral androgen concentrations to drive tumor growth is through the transport of androgens via organic anion-transporting polypeptides (OATPs), which are encoded by *SLCO* genes. In support of this, *SLCO* polymorphisms that encode for more efficient OATP transporters have been associated with worse clinical outcomes [64, 65, 75]. It is notable that statins are a substrate for one of the OATP transporter involved in androgen transport (SLCO2B1), and that in preclinical models statins have been shown to impair DHEAS influx into prostate cancer cell lines. Furthermore, the use of statins in men with advanced hormone-sensitive prostate cancer has been associated with prolonged time to progression—providing the motivation to explore therapeutic strategies aimed at impairing OATP activity [76].

Another potential resistance mechanism, and the focus of several drug development efforts, is the emergence of androgen receptor splice variants (AR-Vs). Many of these AR-Vs retain constitutive activity in spite of lacking the AR ligand-binding domain, which in theory may render drugs that target the ligand–AR interaction (e.g., abiraterone and enzalutamide) ineffective (Fig. 1) [11]. Over 20 AR-V isoforms have been identified from prostate cancer cell lines and clinical samples [17, 77, 78]. The most commonly observed splice variant, known as AR-V7, retains constitutive activity and may be predictive of resistance to both abiraterone and enzalutamide [11, 79]. In a prospective study of 62 patients treated with either enzalutamide or abiraterone, circulating tumor cell AR-V7 mRNA expression was evaluated using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). Patients with detectable AR-V7 transcripts (i.e., AR-V7-positive patients) treated with enzalutamide had significantly lower PSA response rates (0 vs. 53 %, $P = 0.004$) and had significantly shorter overall survival (median

5.5 months vs. not reached, $P = 0.002$). PSA progression-free survival and radiographic progression-free survival were also shortened in AR-V7-positive patients. Abiraterone-treated patients showed similar results, with AR-V7-positive patients having lower PSA response rate (0 vs. 68 %, $P = 0.004$) and shorter overall survival (median 10.6 months vs. not reached, $P = 0.006$). AR-V7-positive patients treated with abiraterone also had shorter PSA progression-free survival and radiographic progression-free survival compared to AR-V7-negative patients [11]. While this association between AR-V7 positivity and resistance to abiraterone and enzalutamide provides a compelling case for AR-V7 as a driver of disease resistance, the possibility remains that AR-V7 may merely signify the presence of a larger resistance program. Additional therapeutic clinical trials are needed to clarify the mechanistic role AR-V7 plays in resistance to AR-directed therapies.

Novel AR-directed approaches to treating CRPC

The inevitable progression of CRPC despite currently available treatments highlights the need for new therapeutic approaches. Past and ongoing efforts to elucidate key mechanisms of resistance to these drugs offer opportunities for identifying new targets. Some of the more promising strategies for targeting persistent AR-signaling in CRPC will be reviewed below (Table 1).

EPI-001 is a small molecule that blocks transactivation of the AR NTD, and has the theoretical advantage over currently available AR-directed therapies of retaining activity in the face of a mutated or truncated AR LBD. It is specific for the AR NTD and does not appear to affect the transcriptional activity of other nuclear steroid receptors [80]. In one LNCaP mouse xenograft model of CRPC, mice treated with EPI-001 had a significant decrease in mean tumor volume compared to control mice [80]. Importantly, EPI analogues have been shown to inhibit the transcriptional activity of constitutively active AR-Vs and to decrease AR-V expressing xenograft growth compared to bicalutamide [81]. It is possible, therefore, that EPI-001 and its analogues may be effective in men with AR-V-positive CRPC.

EPI-001 may also function through mechanism distinct from its ability to bind the AR NTD. It has been shown to inhibit synthesis of the AR in prostate cancer cell lines and fresh prostate cancer tissue cultures at concentrations that inhibit AR target gene expression and prostate cancer cell growth [82]. It also exerts an antitumor effect in AR null lines—potentially through peroxisome proliferator-activated receptor gamma (PPAR-γ) modulation. Additionally, EPI analogues have not been shown to associate with increased AR-FL or AR-V levels, which have been observed with other AR-directed therapies [83]. The EPI-001 analogue, EPI-506, is currently undergoing Phase I/II testing (NCT02606123).

In addition to targeting the NTD of the AR, the DBD of the AR is another potential therapeutic target that should not be affected by alteration in the LBD. The DBD is present in both AR-FL and in most truncated AR-Vs. The DBD is essential to AR's ability to bind ARE and drive downstream transcription. While significant homology exists between the DBD of AR and other nuclear steroid receptors (e.g., glucocorticoid receptor), compounds targeting the AR DBD have been identified that specifically inhibit the AR transcriptional program. Importantly, these agents can diminish AR target gene expression (e.g., *PSA*), including in AR-V-positive preclinical models [84]. Agents targeting the AR DBD are currently in preclinical development.

Instead of directly targeting the AR, AR-signaling can be disrupted by targeting co-regulators of AR transcription. BET bromodomain proteins interact with the NTD of the AR and are thought to affect AR target gene transcription [54]. In castrated mouse models implanted with enzalutamide-resistant prostate cancer cell lines, BET bromodomain inhibitors blocked AR-signaling and BET inhibition in combination with either enzalutamide or ARN-509 in xenograft tumors demonstrated statistically greater antitumor effect. This provides a pre-clinical rationale that BET inhibitors could overcome known mechanisms of enzalutamide resistance, and that, if used in combination with second-generation antiandrogens, may produce more durable responses [85]. There is an ongoing Phase I trial of BET inhibitors in castration-resistant prostate cancer (NCT02705469).

Another novel therapy designed to impair persistent AR-signaling in CRPC is galeterone. Galeterone is a multitargeted agent that has been reported to have a tri-modal mechanism of action. It is reportedly able to inhibit CYP17, acts as an AR antagonist and increases AR protein degradation [86]. Unlike abiraterone, which blocks both 17α-hydroxylase and C17,20-lyase CYP17 isoforms, galeterone more selectively inhibits C17,20-lyase. This may have the beneficial effect of blocking androgen production without leading to the negative feedback loop that drives mineralocorticoid biosynthesis, leading to a number of associated adverse effects (e.g., hypertension, fluid retention and hypokalemia). As such, galeterone may not require the concomitant administration of prednisone, which is typically given in conjunction with abiraterone to prevent the overproduction of mineralocorticoids. In addition to its enzyme inhibitory properties, galeterone also appears to directly antagonize the AR. In LNCaP and VCaP cell lines, galeterone blocked PSA expression, and this blockade was partially reversed by the addition of

Table 1 Select prostate cancer clinical trials

NCT number	Investigational agents	Investigational agent mechanism of action	Disease description	Trial description
01308567	Arm A: Cabazitaxel 20 mg/m^2 plus prednisone Arm B: Cabazitaxel 25 mg/m^2 plus prednisone Arm C: Docetaxel plus prednisone	Taxane chemotherapeutic: microtubule inhibitor	Chemotherapy-naïve mCRPC	Phase III randomized open label
01718353	Arm A: Docetaxel 75 mg/m^2 every 3 weeks + prednisone followed by cabazitaxel 25 mg/m^2 every 3 weeks + prednisone if PSA response to docetaxel <30 % Arm B: Cabazitaxel 25 mg/m^2 every 3 weeks + prednisone followed by docetaxel 75 mg/m^2 every 3 weeks + prednisone if PSA response to cabazitaxel <30 %	Taxanes chemotherapeutic (microtubule inhibitor)	Chemotherapy-naïve mCRPC	Phase II randomized open label
02125357	Arm A: Abiraterone acetate 1000 mg PO daily + prednisone until PSA progression then crossover to Arm B Arm B: Enzalutamide 160 mg PO daily until PSA progression then crossover to Arm A	Abiraterone: CYP17A1 inhibitor, enzalutamide: AR antagonist	mCRPC	Phase II randomized open label
01650194	Enzalutamide daily plus abiraterone daily plus prednisone twice daily	Abiraterone: CYP17A1 inhibitor, enzalutamide: AR antagonist	Bone metastatic CRPC	Phase II single group open-label
02606123	EPI-506	Small-molecule AR NTD inhibitor	mCRPC after treatment with abiraterone or enzalutamide	Phase I/II single group open label
02705469	ZEN003694	Bromodomain inhibitor	mCRPC after treatment with abiraterone or enzalutamide	Phase I single group open label
02438007	Arm A: Galeterone 2550 mg PO daily Arm B: Enzalutamide 160 mg PO daily	Galeterone: CYP17A1 inhibitor and AR antagonist; enzalutamide: AR antagonist	mCRPC with detectable AR-V7 mRNA from circulating tumor cells	Phase III randomized open label
02532114	Niclosamide and enzalutamide	Niclosamide: promotes AR-V degradation; enzalutamide: AR antagonist	mCRPC with detectable AR-V7 mRNA from circulating tumor cells	Phase I single group open label
02003924	Arm A: Enzalutamide 160 mg PO daily Arm B: Placebo	Enzalutamide: AR antagonist	Non-metastatic prostate cancer	Phase III randomized double-blind placebo controlled
02200614	Arm A: BAY1841788 (ODM-201) Arm B: Placebo	AR antagonist	Non-metastatic CRPC	Phase III randomized double-blind placebo controlled
01809691	Arm A: Orteronel Arm B: Bicalutamide	CYP17A1 inhibitor	Metastatic hormone-sensitive prostate cancer	Phase III randomized open label
02445976	VT-464	CYP17A1 inhibitor	CRPC progressing on enzalutamide or abiraterone	Phase II open-label single arm

Table 1 continued

NCT number	Investigational agents	Investigational agent mechanism of action	Disease description	Trial description
02344017	ODM-204	Dual CYP17A1 inhibitor and AR antagonist	mCRPC	Phase I/II open-label single arm
01026623	Cixutumumab and temsirolimus	Cixutumumab: anti-IGF-IR antibody; temsirolimus: mTOR inhibitor	mCRPC	Phase I/II open-label single arm
00683475	Arm A: Ramucirumab + mitoxantrone + prednisone Arm B: Ramucirumab + mitoxantrone + prednisone	Ramucirumab: anti-VEGFR2 monoclonal antibody	mCRPC	Phase II randomized
01322490	Arm A: ProstVac + GM-CSF Arm B: ProstVac + placebo Arm C: Double placebo	ProstVac: poxvirus-based vaccine designed to elicit an immune response to PSA	mCRPC	Phase III randomized double blind
01696877	Arm A: Degarelix prior to prostatectomy Arm B: Cyclophosphamide, GVAX and degarelix prior to prostatectomy	Degarelix: LHRH antagonist; GVAX: prostate cancer cell-based vaccine; cyclophosphamide: alkylating agent	High-risk localized prostate cancer	Phase I/II randomized open label
01341652	Arm A: pTVG-HP vaccine + GM-CSF; Arm B: GM-CSF	pTVG-HP vaccine: DNA vaccine encoding human prostatic acid phosphatase	Non-metastatic hormone-sensitive prostate cancer	Phase II randomized double blind

AR androgen receptor, *mCRPC* metastatic castration-resistant prostate cancer, *NTD* N-terminal domain

DHT, suggesting that galeterone competitively binds to the LBD of the AR [87]. There is also evidence that galeterone degrades the AR and may induce AR-V7 degradation, which indicates that it may be active in AR-V7-positive patients [88–91]. Another multitargeted AR-directed therapy, TAS3681, is reportedly able to antagonize AR activity as well as downregulate its expression in vitro. It is also able to inhibit ligand-independent AR activation in cells that expressed AR-V7 [92].

The ARMOR1 and ARMOR2 trials are open-label Phase I/II studies that were designed to evaluate the safety and efficacy of galeterone in patients with non-metastatic or metastatic CRPC. In ARMOR1, across all treatment doses, 49 % of patients ($n = 49$) achieved a $\geq$30 % decline in prostate-specific antigen (PSA30) and 22.4 % demonstrated a $\geq$ 50 % PSA decline (PSA50). In ARMOR2, across all doses, the PSA30 was 64 % and the PSA50 was 48 % ($n = 52$). Galeterone was well tolerated with the most common adverse events being fatigue, increased liver enzymes, gastrointestinal events and pruritus [86]. Five of six patients with treatment-naive CRPC and high expression of AR-Vs still demonstrated at least a 50 % reduction in PSA following receipt of galeterone, suggesting that it may be able to overcome the resistance conferred by constitutively active AR-Vs [93]. A randomized Phase III trial comparing enzalutamide and galeterone in chemotherapy and abiraterone-naïve mCRPC patients expressing AR-V7 is ongoing (NCT02438007).

In a drug screen attempting to identify inhibitors of AR-Vs, niclosamide, an FDA-approved antihelminthic agent, was found to inhibit AR-V7 in vitro. Mechanistically, niclosamide likely increases AR-V7 protein degradation through a proteasome-dependent pathway, resulting in decreased AR-V7-mediated transcriptional activity. Interestingly, this process appears specific for AR-V7, as preclinical models have not demonstrated that it has an effect on AR-FL expression. As such, niclosamide monotherapy has only modest effect in enzalutamide-resistant prostate cancer xenograft models. However, niclosamide does appear to have excellent synergy when combined with enzalutamide [94]. Niclosamide does have a major limitation in that its oral bioavailability is quite variable, with maximal serum concentrations (C_{max}) following a single 2-g oral dose ranging from 0.25 to 6.0 μg/mL [95]. Fortunately, the lower bound of this C_{max} range still falls within the range of concentrations previously shown to exert an antineoplastic effect on prostate cancer cells, indicating that oral niclosamide may be a viable treatment option for men with mCRPC. A Phase I study testing high-dose niclosamide plus enzalutamide in men with AR-V-positive mCRPC is currently underway (NCT02532114).

Multiple new antiandrogens are also being developed. Apalutamide (ARN-509) is an antiandrogen structurally similar to enzalutamide that demonstrates greater in vivo activity than enzalutamide in xenograft models of CRPC. It underwent Phase I/II clinical trials showing antitumor activity in patients with mCRPC across all doses tested.

The drug was well tolerated, with grade 1–2 fatigue (47 %) and grade 1–2 nausea/abdominal pain (30 %) being the most common adverse effects. It is currently undergoing Phase III testing in men with non-metastatic castration-resistant prostate cancer (SPARTAN trial) [96, 97]. Of note, the PROSPER and ARAMIS trials are testing enzalutamide and ODM-201, respectively (another high-affinity AR antagonist), in similar patient populations (NCT02003924, NCT02200614) [22, 98]. Both of these trials are powered to detect a difference in metastases-free survival—an endpoint previously shown to associate with overall survival [99].

Additional CYP17 inhibitors are also under development. Orteronel (TAK-700) is a non-steroidal antiandrogen that inhibits CYP17. The drug was studied in two Phase III clinical trials for mCRPC (pre- and post-chemotherapy, respectively), but failed to demonstrate a statistically significant improvement in overall survival compared to control [100, 101]. A Phase III Southwest Oncology Group Cooperative Study is currently underway testing orteronel in men with hormone-sensitive, metastatic prostate cancer (NCT01809691). VT-464 is another oral non-steroidal CYP17 inhibitor with greater selectivity for 17,20-lyase. In vitro, it suppresses the androgen receptor axis to a greater extent than abiraterone. In vivo, it decreases intratumoral androgen levels, inhibits tumor growth and decreases PSA with a trend toward statistical significance compared to abiraterone [102]. It also appears to have an effect in spite of AR-V7 expression [103]. Oral VT-464 is currently in Phase II studies to assess tolerability and safety when given second-line to abiraterone or enzalutamide (NCT02445976). Finally, ODM-204 is a novel dual CYP17 and AR inhibitor for the treatment of CRPC that is currently undergoing Phase I/II clinical trials (NCT02344017).

Targeting other oncogenic signaling pathways

While direct targeting of the AR continues to be an active and important area of investigation, non-AR-directed therapies also hold promise (Table 1). Small-molecule inhibitors targeting AR bypass pathways (e.g., PI3K/Akt/mTOR, WNT, JAK/STAT) may be an alternative means of preventing activation of the AR transcriptional program [70]. Alternatively, immunotherapeutic approaches with distinct mechanisms of action from drugs that are designed to impair oncogenic signaling may prove to be effective even in the presence of virulent resistance mechanisms.

A number of small-molecule protein kinase inhibitors have been tested in the CRPC space—all of which have unfortunately failed to demonstrate a clear clinical benefit to date [36, 70]. This is surprising given that many of the pathways being targeted are frequently altered in clinical prostate cancer samples. For example, alterations in the PI3K/Akt/mTOR pathway occur in up to 50 % of CRPC cases, and preclinical models have supported targeting this pathway as a therapeutic strategy [17]. However, efforts to impair PI3K/Akt/mTOR pathway signaling—generally with allosteric mTOR inhibitors—have all failed to date [104–108]. There are a number of reasons the allosteric mTOR inhibitors have not proven effective in spite of an abundance of preclinical data indicating they should be active [106, 109–112]. For one, inhibition of mTOR signaling may lead to a reciprocal upregulation of other oncogenic signaling pathways through a feedback mechanism (e.g., AR, RAS/RAF/MEK) [113, 114]. Allosteric mTOR inhibitors also only inhibit mTORC1, leaving mTORC2 free to activate Akt and drive eIF4E-mediated translation of mTOR-regulated oncogenes [115, 116]. Promising strategies to overcome the shortcomings of the allosteric mTOR inhibitors include the development of: (1) ATP mTOR inhibitors that target both mTORC1 and mTORC2; (2) Akt inhibitors; (3) pan-PI3K inhibitors; and (4) combinatorial strategies targeting multiple signaling pathways.

A variety of agents are also being tested that target other drivers of prostate cancer cell growth, including: insulin-like growth factor-1 (figitumumab and cixutumumab), hepatocyte growth factor inhibitors (rilotumumab), PI3K inhibitors (BKM-120) and notch signaling inhibitors (RO4929097) [36].

Targeting the insulin-like growth factor (IGF) pathway, which has been shown to overlap with the AR and modulate AR-mediated transcription, is the monoclonal antibody cixutumumab. Cixutumumab targets the type 1 IGF receptor (IGF-1R) and has completed through Phase II testing. Unfortunately, in a randomized study testing ADT with or without cixutumumab in men with hormone-sensitive prostate cancer, there was no significant difference in the rate of undetectable PSA at 28 weeks (the primary endpoint) [117]. Other studies examining cixutumumab in combination with mitoxantrone in men with CRPC have shown insufficient activity to warrant further development [118]. However, studies testing cixutumumab combination therapies are ongoing (NCT01026623, NCT00683475).

Hepatocyte growth factor (HGF) and its receptor MET are postulated to play a role in driving CRPC progression. Serum HGF levels are higher in metastatic prostate cancer than in localized tumors and have been associated with worse outcomes [119, 120]. Thus, far drugs targeting HGF or MET have not been successful, however. In a Phase II study of men with CRPC who had progressed on taxane treatment, the HGF inhibiting antibody rilotumumab in combination with mitoxantrone and prednisone failed to demonstrate an overall survival benefit compared to mitoxantrone and prednisone alone (median OS 12.2 vs.

11.1 months, HR 1.10) [121]. Similarly, the dual MET/VEGFR2 inhibitor cabozantinib failed to show an overall survival benefit in the Phase III COMET-1 study, which compared cabozantinib to prednisone alone (median OS 11 vs. 9.8 months, $P = 0.212$). However, given that there is a reciprocal feedback relationship between MET- and AR-signaling, dual targeting of MET and AR may prove to be an effective strategy, and cabozantinib combination studies appear to be warranted [122].

Immunotherapeutic approaches

Immune therapies being studied in prostate cancer include drugs targeting negative co-regulators of T cell activity (i.e., checkpoint inhibitors) and therapeutic cancer vaccines. While a comprehensive overview of the numerous immunotherapeutic approaches being developed for prostate cancer is beyond the scope of this review, we will briefly touch upon a few of the more promising strategies being pursued (Table 1).

Vaccination strategies

Following the success of sipuleucel-T, other therapeutic cancer vaccines have been developed. One of the more promising ones, ProstVac-VF, is a poxvirus-based vaccine targeting PSA. It consists of two poxvirus vectors (vaccinia and fowlpox) that express PSA and the T cell co-stimulatory molecules B7.1, ICAM-1 and LFA-3. ProstVac-VF infects antigen-presenting cells (APCs), which subsequently express PSA and co-stimulatory molecules. These APCs then interact with T cells—resulting in a targeted immune response and T cell-mediated tumor cell death [123, 124]. ProstVac-VF was well tolerated and prolonged overall survival (median overall survival, 25.1 vs. 16.6 months, $P = 0.0061$) in a Phase II trial of men with mCRPC. A Phase III trial testing ProstVac-VF with or without GM-CSF versus placebo in men with asymptomatic to minimally symptomatic mCRPC is ongoing (NCT01322490).

GVAX is a cell-based vaccine that consists of the prostate cancer cell lines PC-3 and LNCaP, which have been engineered to express GM-CSF [125]. Early-phase trials indicated that GVAX had clinical activity, but failed to produce a survival benefit in patients with mCRPC [35, 126]. It is currently being tested as a neoadjuvant therapy prior to prostatectomy in patients without metastatic disease (NCT01696877).

A third vaccination strategy being studied is the use of naked plasmid DNA. These vaccination strategies involve the subcutaneous or intramuscular injection of DNA, which is then taken up by host cells. These cells then express the plasmid-encoded proteins and interact with immune cells to create a targeted immune response. A DNA vaccine encoding prostatic acid phosphatase (pTVG-HP) was shown in the Phase I setting to induce a PAP-specific T cell response [127, 128]. Currently, there is an ongoing Phase II study testing GM-CSF versus GM-CSF plus pTVG-HP in men with biochemically recurrent prostate cancer (NCT01341652).

Checkpoint inhibition

The CTLA-4 and the programmed death 1 (PD1) pathways have both been recognized as clinically important immune checkpoint pathways by which cancers are able to escape T cell-mediated destruction [124]. Disappointingly, a Phase III trial of ipilimumab (a monoclonal antibody that inhibits CTLA-4 activity) following radiation therapy, in patients with docetaxel-refractory metastatic CRPC, failed to show an overall survival benefit when compared with placebo (HR = 0.85, $P = 0.053$) [129]. It is notable that in the subgroup of patients with favorable prognostic features (i.e., alkaline phosphatase $<1.5\times$ upper limit of normal, hemoglobin ≥ 11 g/dL and no visceral metastases), there was a significant improvement in overall survival (HR = 0.62, $P = 0.0038$). While this post hoc analysis does provide some evidence that favorable risk CRPC patients may benefit from ipilimumab, these results ultimately need to be validated in a prospective fashion before ipilimumab can be adopted as a standard for this clinical subgroup of patients.

Another promising immunotherapeutic target is the PD1 immune checkpoint pathway, and thus far inhibitory antibodies directed toward PD1 or its ligand (PDL1) have been shown to produce remarkable clinical responses in a range of malignancies [130–135]. Published experience with anti-PD1/PDL1 therapies in prostate cancer remains limited, however, with only 17 CRPC patients included in the initial Phase I study of nivolumab (anti-PD1)—all of whom failed to respond [136].

One explanation for the low response rate of prostate cancers to immune checkpoint blockade may relate to its relatively low mutational load. Immune recognition is predicated on the presence of antigens that are recognized as foreign (i.e., tumor neoantigens), and it has been recognized that a high burden of tumor neoantigens—as reflected by a high somatic mutational load—associates with response to immune checkpoint blockade [55, 137, 138]. Compared to 'immunoresponsive' tumors like melanoma and non-small cell lung cancer, which have a median of 135 and 147 somatic mutations per tumor, respectively, prostate cancer has a relative low mutational burden (41 somatic mutations per tumor) [138].

Interestingly, low response rates to immune checkpoint blockade have also been documented in patients with

colorectal cancer, which has a median of 66 somatic mutations per tumor [55]. Subsequent analysis of the tumor from a colorectal cancer patient that responded to anti-PD1 therapy revealed that their tumor was mismatch repair (MMR) deficient, resulting in microsatellite instability (MSI) and a high mutational burden (i.e., a *hypermutated* phenotype). Based on the hypothesis that hypermutation would predispose to anti-PD1 therapy, a Phase II study testing pembrolizumab (anti-PD1 therapy) in patients with metastatic carcinoma with and without MMR deficiency (i.e., MSI-high and MSI-low, respectively) was launched [55]. This study demonstrated that 40 % of hypermutated (i.e., MSI-high) colorectal cancer patients had an immune-related objective response (irOR) compared to 0 % of MSI-low patients. Similarly, pembrolizumab was associated with a 50 % response rate in hypermutated non-colorectal gastrointestinal malignancies—supporting the hypothesis that mutational load may predict for response to immune checkpoint blockade in a range of malignancies [134]. While it is likely that other factors (e.g., CTLA-4 and PD1/PDL1 expression) influence immune responsiveness, mutational burden does appear to be a promising predictive biomarker. Importantly, it has been documented that up to 12 % of patients with mCRPC may have a hypermutated genome, which would justify a precision oncology trial to test checkpoint blockade in this molecular subgroup of patients [17, 139].

Conclusion

CRPC remains a vexing and significant clinical problem. Our understanding of the molecular events underlying the progression of prostate cancer to CRPC has evolved, and we now understand that, despite castration levels of serum androgens, the AR pathway remains a central driver of disease progression. With improved understanding comes progress, and as a result of revisiting the AR as a therapeutic target, the treatment landscape for CRPC has changed in the last decade with the FDA approval of two next-generation AR-directed agents (i.e., abiraterone and enzalutamide)—both leading to prolonged overall survival.

The survival benefit of abiraterone and enzalutamide remains modest, however, and more work is needed. The fact that clinical progression on these agents is often heralded by a rising PSA—an AR-regulated gene—highlights that AR-signaling remains a viable target even in the post-abiraterone, post-enzalutamide space. There is an urgent need for new therapies that do not rely on targeting the ligand–AR interaction, but instead function to block this critical oncogenic pathway through different means. To that end, as AR-directed therapies improve and non-AR-mediated resistance mechanisms become more prevalent, it is reasonable to assume that drugs not directly targeting the AR (e.g., small-molecule inhibitors targeting other oncogenic pathways or immunotherapies) will be needed. Fortunately, our understanding of this disease has continued to improve, and as highlighted above, there are a number of very promising therapeutic strategies making their way into the clinic.

Compliance with ethical standards

Conflict of interest None.

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