Targeting the androgen receptor in metastatic castrate-resistant prostate cancer: A review

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Abstract

Despite recent advances in the treatment of advanced prostate cancer (PCa), metastatic castrate-resistant PCa remains incurable at this time. The androgen receptor (AR) plays a key role in the development and progression of PCa, continuing to be active in most patients even after the development of castration resistance. Here, we aim to more closely review the mechanisms by which AR signaling is maintained, including AR overexpression/overamplification, intracrine androgen synthesis, AR mutations, and the development of AR splice variants. We also review therapies targeting each of these mechanisms. We also discuss the potential role of AR-CAG repeats and AR splice variants as potential biomarkers of response to hormonal manipulation therapies. Published by Elsevier Inc.

Keywords: Castration-resistant prostate cancer; Androgen receptor

Introduction

Prostate cancer (PCa) is currently the second most common cancer affecting men in the world [1]. It is estimated that there will be approximately 220,800 new cases diagnosed, and 27,540 deaths from PCa in 2015 in the United States alone [2]. Most patients with PCa are diagnosed with localized disease. Management at this stage includes radical prostatectomy, radiation therapy, active surveillance, or combined approaches including concurrent hormonal therapy with radiotherapy. The 5-year overall survival in patients with localized or regional disease is excellent, approaching 100%. However, for patients with metastatic disease, the prognosis is dramatically different, with an estimated 5-year overall survival of 28% [2]. Management at the metastatic stage requires systemic therapy most commonly using hormonal, chemotherapeutic, immunotherapeutic manipulation, or a combinatorial approach.

PCa growth and proliferation is primarily dependent on androgens, and androgen deprivation therapy (ADT) is an effective means of controlling the disease. Eventually, however, all men develop resistance to androgen deprivation, resulting in the development of castration-resistant PCa (CRPC). CRPC remains the lethal form of PCa. Although, to some, the term CRPC may connote a “hormonally refractory” state, recent studies have shown that further hormonal manipulation can result in impressive disease control even after progression on ADT, and thus, many patients with CRPC would respond to further hormonal manipulation [3–5].

To best understand how CRPC can respond to further hormonal interventions, it is important to recognize that the development of PCa and CRPC results from a multistep process in which androgen receptor (AR) signaling plays a key role. For most patients, AR signaling remains the primary oncogenic driver despite castrate testosterone levels, and its activation has been observed to be mediated through a multitude of mechanisms [3,6,7]. In this article, we review the major mechanisms through which AR signaling is sustained, including AR gene amplification and overexpression, AR mutations, constitutively active AR splice variants, and intratumoral androgen synthesis. Lastly, we explore the role of the emerging field of CAG repeats within the AR gene and its influence on oncogenesis and disease progression.
The AR

AR is located on chromosome Xq11–13 and is a ligand-dependent transcription factor with multiple functional domains. The NH2-terminal domain (NTD) acts as a transcriptional activation domain responsible for the most AR transcriptional activity. The central domain of the AR is the DNA binding domain, made up of 2 zinc-finger motifs. This is followed by a short, flexible, hinge sequence responsible for nuclear localization upon activation. The COOH-terminal domain of the AR is home to the ligand binding domain (LBD) and comprises the remainder of the AR transcriptional activity [8]. In the absence of androgens, AR remains bound in an inactive state in the cytoplasm by heat shock proteins. Upon ligand binding, AR undergoes a conformational change exposing its nuclear localization hinge region. This prompts translocation of the bound complex to the nucleus [9,10]. Within the nucleus, the AR DNA binding domain interacts with androgen-response elements to recruit transcriptional coregulators and begin transcription [9,11].

In normal tissue, transcription of these downstream genes helps maintain appropriate architecture and physiologic function of the prostate. However, in PCa, repetitive transcription of these downstream targets serves to promote cancer cell survival and proliferation. One such gene product is prostate-specific antigen (PSA), which, although not directly linked to cell survival, is nonetheless a helpful serum biomarker to monitor disease activity.

Existing hormonal therapies have been developed with the aim of decreasing circulating androgens to decrease AR signaling and decrease PCa cells’ ability to thrive. This ADT is typically achieved either by orchietomy or, more commonly, medical castration with leutinizing hormone-releasing hormone therapy.

Although surgical or medical castration initially works in the vast majority of patients with PCa, the cancer eventually develops resistance to ADT. Although resistance to ADT was initially thought to represent a hormone-refractory state, recent evidence indicates androgen signaling is crucial to the survival of most CRPC cells. Here, AR signaling is preserved and sustained through AR overexpression or overamplification, intracrine androgen synthesis, AR mutations, and other aberrant signaling patterns, including the development of AR splice variants (Fig.). This review takes a deeper look at each of these mechanisms to better understand how androgen signaling is maintained in the castration-resistant state, and to discuss novel therapies that target this aberrant signaling. It should be noted that there are a number of new therapies approved or in development including immunotherapy (Sipuleucel T, ProstVac, and Iplulumab), radiopharmaceuticals (Radium-223), and chemotherapy (docetaxel and cabazitaxel). Although each of these has activity in advanced PCa, none specifically targets AR signaling as its sole mechanism of action, and would therefore not be covered in this review.

AR overexpression and intratumoral androgen synthesis

The term CRPC is relatively recent. In past years, PCa that progressed despite ADT was described as “androgen-independent” PCa or “hormone-refractory” PCa, implying that tumor growth occurred via completely androgen-independent pathways. In 1997, Koivisto et al. evaluated AR gene amplification and mRNA expression in the tumors of 54 men who had failed primary ADT. A total of 26 of these patients had paired primary tumor samples available for analysis. Approximately 30% of the “therapy-resistant” tumors exhibited both wild-type AR gene amplification and substantially elevated AR mRNA levels, raising the possibility that androgen signaling was still playing an important role. Interestingly, the primary tumor in these patients, and untreated patients, did not exhibit AR gene amplification, suggesting evolution of these tumors over time in response to castrating therapy [12,13]. Multiple studies since this landmark study have confirmed increased levels of AR mRNA, AR protein, and amplification of the AR gene in CRPC tumors [12,14–19]. This elevation in AR copy number is thought to help increase AR sensitivity to the low levels of circulating androgens present in the castrate setting to maintain AR signaling. A possible scenario for this evolution is that most primary tumor cells respond to ADT, however, a small pre-existing population of cells with amplified AR are selected based on their ability to grow in the castrate environment and thereby create a clonal population of tumor cells that are able to maintain AR signaling. An alternative hypothesis posits that AR amplification and overexpression evolves in AR copy-normal cells in response to castrating therapy, conferring a survival advantage in the androgen-depleted environment, and clonal selection then proceeds in a Darwinian-like manner.

Another documented mechanism through which tumors can acquire resistance to ADT and increase sensitivity to the low level of circulating androgens available after ADT is via local, intratumoral autocrine androgen synthesis [20–22]. In a study by Page et al., 13 men with PCa received ADT and were compared with patients receiving a placebo control, and all had intraprostatic androgen levels measured. The men receiving ADT showed 94% reduction in serum testosterone, but only a 70% and 80% respective decrease in intraprostatic levels of testosterone and dihydrotestosterone (DHT) [22]. DHT, the active metabolite of testosterone, is synthesized through enzymatic reduction via 5α-reductase, and is known to be a more potent androgen than testosterone. CRPC has been found to overexpress 5α-reductase, suggesting that the tumor attempts to increase sensitivity to androgens by converting testosterone to its more potent form of DHT [21]. Intratumoral androgens are also synthesized from precursors such as cholesterol and dehydroepiandrosterone, similar to their synthesis in the adrenal gland. Compared to their primary counterparts, CRPC tumors
were found to have increased expression of steroidogenic enzymes, including CYP17A1 [21,23].

**Targeting androgen synthesis**

AR amplification/overexpression and increased intratumoral androgen synthesis serve to increase the sensitivity of AR to the lower levels of circulating androgens present after primary ADT. This mechanism of resistance allows tumors to continue using their main signaling pathway (AR signaling) to grow in a castrate environment. This understanding has helped spur the development of a number of therapies given after the failure of initial castrating therapy that actively target these mechanisms.

**Ketoconazole**

Ketoconazole is perhaps the oldest of these “secondary hormonal therapies” that decrease AR signaling in the castrate environment. Ketoconazole is an antifungal drug designed to disrupt fungal cell wall synthesis by inhibiting multiple enzymes involved in cholesterol metabolism. Interestingly, in humans, cholesterol is the major building block for adrenal hormones including cortisol, aldosterone, and the androgens. A major side effect of ketoconazole, therefore, is adrenal insufficiency, which is due to inhibition of multiple CYP enzymes involved in cortisol and androgen synthesis, including CYP17 [24]. Therapeutically, ketoconazole was evaluated in a large, randomized phase III trial lead by Small et al. This study evaluated the utility of antiandrogen withdrawal therapy vs. ketoconazole in men with newly diagnosed CRPC and showed a significant difference in PSA response ($P = 0.002$) between those on ketoconazole (27%) vs. antiandrogen withdrawal therapy alone (11%), indicating activity of this agent in PCa [25].

**Abiraterone acetate**

More recently, abiraterone acetate was specifically developed to act as a potent, selective inhibitor of CYP17A1, which converts pregnenolone and progesterone to 17OH-Pregnenolone and 17OH-Progesterone, respectively, during androgen biosynthesis. Unlike ketoconazole, abiraterone does not affect other CYP enzymes, which is thought to lead potentially to improved efficacy (although the 2 have never been compared in a head-to-head clinical trial) and less off-target effects [26]. Notably, while abiraterone dramatically decreases androgen synthesis, blockade of CYP17 also decreases cortisol synthesis, and like ketoconazole, leads to adrenal insufficiency. Initial
development of abiraterone acetate was stymied by both this and compensatory mechanisms leading to increased mineralocorticoid synthesis. This hurdle was overcome in clinical trials through the addition of low-dose prednisone (5–10 mg daily) to abiraterone acetate, which both replaces the lost cortisol and abrogates the mineralocorticoid excess.

Abiraterone was shown to have activity including dramatic PSA declines and objective radiographic responses in phase I and phase II studies [27–29]. A randomized phase III study in patients with docetaxel-refractory metastatic CRPC (mCRPC) (COU-AA-301) compared abiraterone plus prednisone with prednisone alone, and showed increased median overall survival (15.8 mo [95% CI: 14.8–17.0] vs. 11.2 mo [10.4–13.1]; hazard ratio [HR] = 0.74; 95% CI: 0.64–0.86; \(P < 0.0001\)), progression-free survival (8.5 mo, 95% CI: 8.3–11.1, in the abiraterone group vs. 6.6 mo, 5.6–8.3, in the placebo group; HR = 0.63; 0.52–0.78; \(P < 0.0001\)), and PSA response (235 [29.5%] of 797 patients vs. 22 [5.5%] of 398; \(P < 0.0001\)) in patients treated with abiraterone. A second phase III trial in patients with docetaxel-naive CRPC (COU-AA-302) led by Ryan et al. [5,32], revealed similar benefits of increased overall survival (34.7 mo [95% CI: 32.7–36.8] vs. 30.3 mo [28.7–33.3]; HR = 0.81 [95% CI: 0.70–0.93]; \(P = 0.0033\)) with abiraterone therapy.

**Orteronel, galeterone, and VT-464**

Orteronel (formerly TAK-700) was designed to be a CYP17A inhibitor with stronger selectivity for inhibition of 17, 20-lyase with the goal of reducing the off-target effect on mineralocorticoid production compared with that of abiraterone acetate. In a phase I/II dose escalation trial, orteronel strongly suppressed testosterone production and PSA declines of greater than 50%, which were observed in approximately 52% of patients [33]. This trial was done without prednisone supplementation given its specificity in mechanism of action. However, adrenocorticotropic hormone stimulation tests demonstrated blunted responses in patients, suggestive of impaired cortisol production, implying that a low dose of prednisone would be required in further studies.

Despite early optimism, an interim analysis of data from a phase III trial of orteronel plus prednisone vs. prednisone alone in patients with mCRPC, who progressed after chemotherapy (ELM-PC 5) suggested that the trial would not meet its primary endpoint of increasing overall survival and the trial was halted [34]. Whether this was because of the absence of biologic activity of orteronel in CRPC or because of the fact that a substantial number of patients in the control arm subsequently received abiraterone acetate remains unclear. A phase III trial (ELM-PC 4) evaluating orteronel plus prednisone vs. prednisone in the chemotherapy-naive population was recently published, and although it did not demonstrate a significant improvement in overall survival, a significant improvement in radiologic progression-free survival was observed. The median radiographic progression-free survival was 13.8 months (95% CI: 13.1–14.9) with orteronel plus prednisone and 8.7 months (8.3–10.9) with placebo plus prednisone (HR = 0.71; 95% CI: 0.63–0.80; \(P < 0.0001\)) [35]. The median overall survival was 31.4 months (95% CI: 28.6–not estimable) with orteronel plus prednisone and 29.5 months (27.0–not estimable) with placebo plus prednisone (HR = 0.92; 95% CI: 0.79–1.08; \(P = 0.31\)) [35]. Based on these phase III studies, the development of orteronel in mCRPC has been discontinued, although it is currently under evaluation in a Southwest Oncology Group randomized study of ADT plus bicalutamide vs. ADT plus orteronel in men with newly diagnosed, hormone-sensitive, metastatic disease (Southwest Oncology Group 1216).

Galeterone is designed to be a potent antiandrogen agent that targets the following 3 different blocking androgen signaling: CYP17 inhibition, direct AR inhibition, and AR degradation via ubiquitin-mediated mechanisms. The phase I (ARMOR1) trial demonstrated that 22% of patients treated with galeterone (without prednisone supplementation) had PSA declines of >50% [36]. Results from the phase II (ARMOR2) study have recently been presented at European Society for Medical Oncology and American Society of Clinical Oncology meetings. The best response was demonstrated in patients with metastatic disease, who were otherwise treatment naive (\(n=36\)). PSA declines of 30% and 50% were achieved in 89% and 81% of patients, respectively [37]. Excitingly, inhibition of CRPC tumors harboring putative splice variants (to be discussed later) was observed in analysis of circulating tumor cells (CTCs) taken from patients under ARMOR2 study. In this analysis, an AR C-terminal truncation in CTCs was hypothesized to indicate the presence of AR splice variants, specifically AR-V7 [37]. PSA declines of >50% were observed in all 4 patients harboring C-terminal truncations, suggesting activity of galeterone in patients harboring splice variants. This is an intriguing finding as recent work has suggested that neither abiraterone acetate nor enzalutamide have significant activity in this population [38]. Based on this observation, galeterone would be compared to enzalutamide in a phase III study randomizing men with mCRPC with identified AR-V7 variant.

VT-464 is another CYP17 inhibitor more specific for 17, 20-lyase, which showed promise in preclinical studies [39,40]. The phase I trial presented at American Society of Clinical Oncology genitourinary symposium in 2015 demonstrated 19 of 26 patients had at least 30% reduction in PSA [41]. Interestingly, the preliminary results and some preclinical data suggest stronger potency in patients previously treated with abiraterone or enzalutamide [40,41]. Phase 2 studies are currently ongoing at this time.

Of note, AR amplification may itself serve as a biomarker of clinical outcomes. A recent study published by Azad et al. [42] used cell-free DNA analyses from patients treated with either enzalutamide or abiraterone to
demonstrate that patients with an AR gene aberration, defined as copy number variation or mutation in exon 8, had poorer clinical outcomes, lower rates of PSA decline, and shorter time to progression. Another study by Salvi et al. [43] demonstrated similar results when looking at AR copy number variation and mutations in the CYP17A gene in patients treated with abiraterone.

**AR mutation and splice variants**

Like many other receptors, the AR has a certain level of promiscuity. This is often enhanced or diminished by mutations within the gene, allowing for activation by weaker androgens such as dehydroepiandrosterone, estrogens/progesterone, or even cortisol [44,45]. It has been well established that the presence of mutations within AR is more prominent in advanced CRPC compared to primary tumors, conferring a survival advantage for these cells [8,44,46–49]. A database has been created identifying a number of substitutions occurring in AR (http://androgendb.mcgill.ca/), some of which have identifiable consequences. Mutations can result in the conversion of AR antagonists (bicalutamide, nilutamide, and flutamide) to agonists [50,51]. More recently, Azad et al. [42] identified novel mutations conferring resistance to enzalutamide (F876L) and abiraterone (H874Y and T877A). A few mutations, including the T877A mutation, have been shown to constitute activating AR. Others occurring at the NTD or in the DNA binding domain, alter the binding specificity of coregulators promoting transcriptional activation of downstream genes [52–55]. Truncated forms of AR, lacking its carboxy-terminal region, seem to confer a paracrine effect generating clonal cooperation with neighboring PCA cells, possibly aiding in both invasion and metastatic potential of the tumor [54].

**Antiandrogens: Bicalutamide, flutamide, nilutamide, and enzalutamide**

Antiandrogens are a class of drugs, which have been established as potent therapeutic agents in the treatment of PCA for over 40 years [56]. They bind to the LBD of the AR through competitive inhibition of testosterone and the more potent DHT. Flutamide was the first antiandrogen approved for use in management of advanced PCa by the late 1970s/early 1980s [57–59]. Bicalutamide was developed thereafter and found to be significantly more potent than flutamide with a much improved side effect profile, making it the preferred antiandrogen by the mid-1990s [60,61]. Nilutamide was developed around the same time and was shown to be relatively well tolerated as well [62]. More recently, enzalutamide has joined, and possibly superseded this class of agents. Enzalutamide has been shown to have more than 5-fold greater affinity for AR than bicalutamide and works via 2 different mechanisms of action. In addition to competitive inhibition of the AR, enzalutamide impairs AR nuclear localization and has been shown to cause a conformational change in AR impairing DNA binding and cofactor recruitment [63]. After promising data from phase I and phase II studies [64], the phase III AFFIRM trial comparing enzalutamide to placebo in the postchemotherapy setting revealed significant improvement in overall survival during interim analysis; the median overall survival was 18.4 months (95% CI: 17.3 to not yet reached) in the enzalutamide group vs. 13.6 months (95% CI: 11.3–15.8) in the placebo group (HR for death in the enzalutamide group, 0.63; 95% CI: 0.53–0.75; \( P < 0.001 \)) [65]. The phase III PREVAIL trial followed soon thereafter evaluating enzalutamide vs. placebo in the prechemotherapy setting in patients with mCRPC. Interim analysis, again, showed significant improvement in overall survival, and significant improvement in radiographic progression-free survival at 12 months—65% of those treated with enzalutamide compared with 14% for patients who received placebo (81% risk reduction; HR in the enzalutamide group, 0.19; 95% CI: 0.15–0.23; \( P < 0.001 \)) [4].

In a similar study, the TERRAIN trial compared enzalutamide to bicalutamide in patients with mCRPC, who were receiving ADT. In patients with measurable soft tissue masses, objective tumor response rates were 54% in patients taking enzalutamide, and 11% in patients taking bicalutamide [66]. As with bicalutamide, there is a concern that enzalutamide could potentially cause a tumor flare after withdrawal of the agent because of compensatory increase in testosterone. Long-term follow-up is still ongoing at this time.

Outside the CRPC setting, a single-arm, phase II study was conducted to evaluate the use of enzalutamide as monotherapy instead of ADT. Response rates, determined by PSA decline and radiographic response were consistent between ADT (>80%) and enzalutamide (92%). Short-term adverse events were comparable as well [67]. Although intriguing, these data do not yet support the routine use of enzalutamide monotherapy in place of ADT, and further clinical trials are needed to demonstrate comparability in terms of long-term outcomes.

The fact that both enzalutamide and abiraterone are well tolerated and are both approved for men with mCRPC raises the question of whether combination therapy is better than sequential therapy. To this point, there has been only modest activity observed of enzalutamide in men with abiraterone-refractory disease, and similarly of abiraterone in men with enzalutamide-refractory disease. To determine whether combined targeting of the AR would provide synergistic clinical activity compared with monotherapy, there is an ongoing open-label, randomized phase III study conducted by the Alliance for Clinical Trials Cooperative Group evaluating enzalutamide vs. enzalutamide in combination with abiraterone (NCT01949337).
**Newer antiandrogens: ARN-509 and ODM-201**

Other AR antagonists are currently under evaluation. ARN-509 is a selective AR antagonist, lacking agonistic activity. It has demonstrated greater specificity and potency than enzalutamide in preclinical studies [68]. Preliminary results from the phase II portion of the phase I/II trial revealed an excellent response rate, with 88% of men with mCRPC, who are chemotherapy- and abiraterone-naive experiencing PSA declines of greater than 50%; 29% percent of men with mCRPC pretreated with abiraterone experienced a similar response. ARN-509 appears to be overall very well tolerated [26,69] with mostly hormonal side effects similar to enzalutamide, and less commonly gastrointestinal side effects. Studies of ARN-509 in combination with abiraterone acetate and other compounds are currently ongoing. A phase I study of ARN-509 given with abiraterone acetate has shown that the combination is well tolerated [70]. A phase III randomized, placebo-controlled, double-blind study of ARN-509 in combination with abiraterone in men with chemotherapy-naive mCRPC is set to enroll patients in 2015 (NCT02257736). There is also a phase III study (SPARTAN) ongoing to evaluate metastasis-free survival using ARN-509 in combination with ADT vs. ADT given with placebo (NCT01946204) in men with non-metastatic CRPC. ARN-509 is also under investigation as monotherapy or in combination with Lupron for men with biochemical recurrence after surgery or radiation, to see if quality of life and metabolic side effects, including changes in bone mineral density, cholesterol, and whole-body muscle and fat composition are better compared with those of Lupron monotherapy (NCT01790126).

ODM-201 is another AR antagonist currently being evaluated in phase I/II trials. This is a new generation AR inhibitor developed specifically to target CRPC. Its structure is distinct from enzalutamide and has a high binding affinity, which prevents AR nuclear localization. Preclinical studies suggest this may have a higher affinity for binding AR than bicalutamide, enzalutamide, and ARN-509, with excellent potency in VCaP cells [71,72]. The ARADES trial evaluated its safety/tolerability and response rate in an open-label phase I/II trial. The results suggested ODM-201 monotherapy in patients with mCRPC may lead to disease suppression (29%–33% of men in all dosage arms had PSA declines of greater than 50% at 12 wk) [71]. Table 1 summarizes major phase III trials investigating secondary hormonal agents discussed above.

**AR splice variants**

Alternative splicing is a regulated process in healthy cells, which allows a single gene to code for multiple proteins by using different combinations of exons and introns during gene expression. Splice variants are active mRNA products resulting from alternative splicing. Several AR splice variants have been identified, some with significant clinical implications. Whether these have a role in normal AR physiology is not well understood. Approximately 20 different AR splice variants have been identified in PCa cell lines, models, and clinical tumors [73]. Splice variants have drawn more attention for their clinical relevance in recent years when several were discovered to lack the LBD compared with full length AR (AR-FL) [73,74]. Lacking the binding domain for androgens suggested that these variants could function in an androgen autonomous fashion, and in vitro studies had suggested these truncated variants could potentially have constitutive activity and AR function [75].

This hypothesis was soon confirmed in a series of studies between 2008 and 2010, which identified most AR variants achieved through alternative splicing and cryptic exons [76–79]. Of these variants, AR-V1, AR-V7/AR3, AR-12/AR V567es, and AR-V9 were found to have the most putative clinical relevance. The mRNA expression of AR-V1 and AR-V7 were found to be significantly higher in CRPC compared with that in hormone-naive PCa [77]. Transcript levels of AR-V1, AR-V7, and AR V567es were also found to be significantly higher in analyses of CRPC bone metastases compared with those of hormone-naive tumors [80]. AR-V1 and AR-V9 were found to be mainly cytoplasmic and were described to be conditionally active, rather than constitutively active, as they exhibited ligand-independent activity in some cell lines but not in others [81,82]. AR-V7 and AR V567es, however, are constitutively active and consistently exhibit nuclear localization in an androgen-independent manner [77,83]. Of these 2 splice variants, more work has been performed analyzing the role of AR-V7 compared with that of AR V567es since it has been possible to develop a variant-specific antibody and complementary sequences, which target the AR-V7 variant.

AR splice variants are thought to confer a mechanism of resistance to both primary and secondary ADT. There is an increasing evidence that these splice variants can work in conjunction with full length AR proteins to potentiate AR signaling, even in the presence of potent antiandrogens such as enzalutamide [84]. Although AR variants have been shown to bind to their target DNA sequences without AR-FL, in the presence of AR-FL, they have been shown to co-occupy the canonical AR targets with AR-FL in a mutually-dependent manner. This suggests that AR variants are capable of controlling the degree of response of AR-FL to androgen-directed therapy by activating AR-FL in an androgen-independent manner [84].

Clinically, the presence of AR splice variants has generated significant interest. A prospective study recently published by Antonarakis et al. [38] investigated the correlation between AR-V7 expression in CTCs and treatment response of patients with CRPC treated with either enzalutamide or abiraterone. Approximately 39% of men treated with enzalutamide and 19% of men treated with abiraterone were identified to harbor the AR-V7 variant in...
### Selected important completed and ongoing phase III trials of secondary hormonal agents in the treatment of prostate cancer

<table>
<thead>
<tr>
<th>Experimental arm</th>
<th>Control arm</th>
<th>Name of the study</th>
<th>Clinical trials identifier</th>
<th>Clinical state</th>
<th>Prior chemotherapy</th>
<th>Overall survival</th>
<th>Progression-free survival</th>
<th>PSA response</th>
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<td>Placebo + prednisone</td>
<td>COU-AA-301</td>
<td>NCT00638690</td>
<td>mCRPC</td>
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<td>15.8 mo vs. 11.2 mo ($P &lt; 0.0001$; HR = 0.74)</td>
<td>8.5 mo vs. 6.6 mo ($P &lt; 0.001$; HR = 0.63)$^a$</td>
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<td>No</td>
<td>34.7 mo vs. 30.3 mo ($P = 0.0033$; HR = 0.81)</td>
<td>16.5 mo vs. 8.3 mo ($P &lt; 0.001$; HR = 0.53)$^a$</td>
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<td>13.8 mo vs. 8.7 mo ($P &lt; 0.0001$; HR = 0.71)</td>
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<td>PREVAIL</td>
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<td>At interim analysis, estimated: 32.4 mo vs. 30.2 mo ($P &lt; 0.001$; HR = 0.73)</td>
<td>At 12 mo: 65% vs. 14% ($P &lt; 0.001$; HR = 0.19)$^a$</td>
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$^a$Use of radiographic data to determine progression. Remainder of data reports PSA progression.
$^b$Ongoing trials.
CTCs. Patients considered positive for AR-V7 had dramatically lower PSA response rates (0% vs. 53%; \( P = 0.004 \)), shorter median PSA progression-free survival (1.4 mo vs. 6.0 mo; \( P < 0.001 \)), shorter median clinical or radiologic progression-free survival (2.1 mo vs. 6.1 mo; \( P < 0.001 \)), and shorter median overall survival (5.5 mo vs. not reached; \( P = 0.002 \)). Similarly, among men receiving abiraterone, patients considered positive for AR-V7 had lower PSA response rates than patients considered negative for AR-V7 (0% vs. 68%; \( P = 0.004 \)), shorter median PSA progression-free survival (1.3 mo vs. not reached; \( P < 0.001 \)), shorter median clinical or radiologic progression-free survival (2.3 mo vs. not reached; \( P < 0.001 \)), and shorter median overall survival (10.6 mo vs. not reached; \( P = 0.006 \)) [38].

Although these are exciting early data, overall, this study requires confirmation by a large scale effort with biopsy correlation. If confirmed, the presence of AR-V7 in CTCs could serve as a predictive biomarker to help clinicians decide which patients are likely to benefit from further hormonal therapy. Of note, a recent study by the same group showed that the presence of AR-V7 does not confer resistance to cabazitaxel chemotherapy, implying that AR-V7 expression in CTCs may serve as a predictive biomarker for response to hormonal therapy, as opposed to a more basic marker of poor prognosis [85].

This compelling evidence suggesting AR-V7 may confer resistance to available hormonal therapies has led to research attempting to identify agents that specifically target splice variants or the AR NTD. Unfortunately, the NTD proves to be a challenging target for drug design given its flexibility with high degree of intrinsic disorder. The AF-1 region of the NTD is known to contain most AR transcriptional activity and characteristically has collapsed disorder, allowing for some secondary structure without a stable tertiary structure [86]. The small molecule inhibitor EPI-001 was found to interact with the AF-1 region, thereby attenuating its activities by inhibiting protein–protein interactions with AR, and reducing AR interaction with the androgen-response elements on its target genes [86,87]. Preclinical studies have demonstrated that EPI-001 inhibits AR-dependent proliferation in human PCA cells. In mice models with PCA xenografts, EPI-001 injections blocked the growth of the xenograft regardless of the presence of androgen. However, it had no effect on models lacking functional AR, suggesting that his drug only affects cells dependent on AR for growth and proliferation [86,88]. Challenges in translating this agent from the laboratory into an orally bioavailable agent has hampered its development and, therefore, at this time, clinical trials are not yet underway.

Specifically targeting AR-V7 has become an area of interest. As mentioned previously, there is exciting evidence to suggest galeterone may target AR-V7 expressing tumors possibly by potentiating ubiquitin-mediated degradation of the variant AR protein [37]. A study shows that niclosamide, an antihelminthic teniacide, may be a potent inhibitor of AR-V7 in PCa cells by significantly down-regulating AR-V7 protein expression through increased protein degradation in a proteasome-dependent pathway [89]. The study demonstrated compelling evidence that niclosamide inhibited PCa growth in in vitro and tumor growth in vivo models. Furthermore, it was shown that this strategy may overcome or minimize enzalutamide resistance. Using a combination of enzalutamide with niclosamide in preclinical models was shown to significantly inhibit enzalutamide-resistant tumor growth. This work needs further validation in clinical trials, but appears promising in an era of enzalutamide and abiraterone-resistant CRPC. Other AR splice-variant inhibitors are currently in development [83].

CAG repeats

Repetitive CAG sequences are present in exon 1 of the AR. They are highly polymorphic and encode long homopolymeric amino acid chains in the NTD of the AR gene [90]. Shorter CAG repeat length has been observed to correlate with a higher androgen binding affinity and higher receptor transactivation activity [90,91]. Based on this finding, it has been suggested that CAG repeat length may correlate with clinical outcomes. Specifically, in recent years there has been a debate regarding whether there is a clear association between the number of CAG repeats within the AR and an increased risk of developing PCa. A large review of case series performed in 2004 to include over 4,000 patients by Zeegers et al. [92] suggested a correlation between short CAG repeat length and increased risk of developing PCa. Since then, 2 large, nested case–control studies from the Prostate Cancer Prevention Trial (2010 and 2014) did not find any significant associations between CAG repeat length and the risk of developing PCa [93,94]. Interestingly, some studies did identify consistent CAG repeat lengths within ethnic groups [90,95,96], with work showing that African–American men have shorter CAG repeats. These short CAG repeats are more often associated with higher transactivational function, which could offer an explanation for the increased incidence of PCa in this population. Short CAG repeats in Japanese men appear to have prognostic value in predicting longer responses to hormonal therapy [90,96]. Other studies have been performed attempting to determine whether CAG repeat length would be a valuable biomarker or prognostic tool, however, to date, no significant correlation has been established [97–99]. What the effect of CAG repeat length is at the time of castration resistance, and whether this influences either response to subsequent hormonal therapy, or the development of AR splice variants is unknown, but may be a future research direction.

Southwell et al. suggested that AR mutations may alter the inverse relationship between CAG repeat lengths and the transactivation in a minor way, which increased N/C-terminal interactions. The common T877A mutation is
known to increase LBD promiscuity allowing more ligands to activate AR. When this mutation is present, this study found that men with shorter CAG repeats no longer have the transactivation pattern otherwise associated with the mutation [100]. This suggests that certain mutations within the AR could possibly override the effect AR-CAG repeat length may have in PCAs. Further studies to identify the presence of mutations and AR-CAG repeat length may help reveal a more conclusive association of CAG repeat with incidence of PCa or to be used as a possible prognostic tool in the presence of certain mutations. Although the data appear inconclusive at this time, with some studies showing suggestive associations, some refuting these associations, and some simply equivocal [90,92–96,101,102], further investigation may be warranted.

Conclusion

Huggins and Hodges radically changed the field of PCa when they first described androgens as the major drivers of PCa more than 75 years ago. Although our understanding of PCa and the role of the AR has substantially changed since then, the AR continues to remain a major driver in the growth and survival of PCa, including in CRPC. This is supported by the numerous mechanisms of resistance that tumors develop to maintain AR signaling despite more effective and potent ADTs. Clinically, novel AR-targeting therapy, including abiraterone acetate and enzalutamide, have resulted in excellent response rates when used in conjunction with ADT at the time that CRPC develops. However, these agents are not a cure for PCa, and recent data has shown significant cross-resistance, implying shared mechanisms of resistance (e.g., splice variants). Currently, a number of clinical trials are ongoing to determine whether there is improved efficacy when these AR-targeting therapeutics are used in combination rather than sequentially as monotherapy, and to establish reliable predictive biomarkers that can guide treatment decisions. Novel therapies, such as galeterone, EPI-001, and niclosamide, which exploit established mechanisms of resistance, would hopefully translate to potent therapies in the clinical setting. Although there is mounting evidence that CRPC may develop neuroendocrine differentiation to function in an AR-independent manner [103], there is some data to suggest that AR expression is persistent even in these tumors [104], and further studies need to be done to determine the sensitivity of neuroendocrine-like PCa tumors to AR manipulation. Although we have made remarkable progress in further understanding the biology of PCa since the discovery of its androgen dependence 75 years ago, the ever-evolving nature of PCa poses both challenges and opportunities to researchers trying to understand new mechanisms of resistance and develop novel therapeutics.

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