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Seminar article

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.

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The AR

AR is located on chromosome Xq11–13 and is a ligand-dependent transcription factor with multiple functional domains. The NH_2-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 orchiectomy or, more commonly, medical castration with leuteinizing 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 Ipilumumab), 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-exisiting population of cells with amplified ARare 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

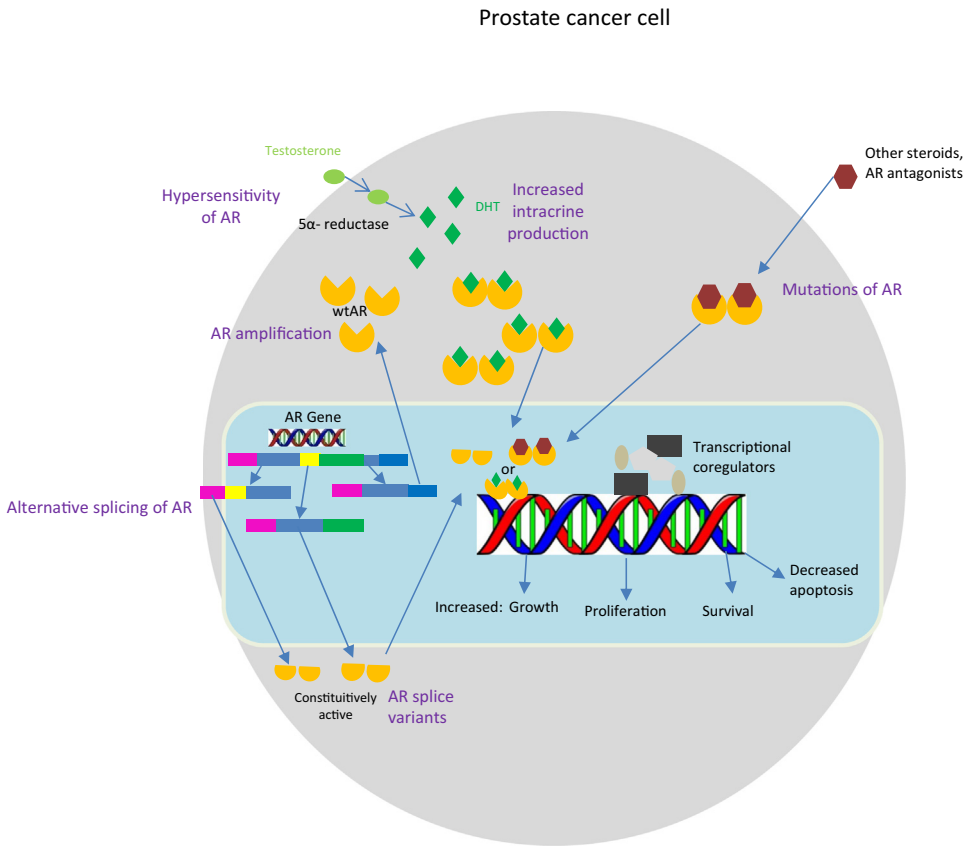

Fig. Depiction of mechanisms of resistance to androgen deprivation therapy addressed in this review: AR amplification, increased intratumoral androgen synthesis, hypersensitivity of AR, mutations of AR, and alternative splicing of AR to constitutively active splice variants. (Color version of figure is available online.)

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 ketoconzole, 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 CPRC (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; $P < 0.0001$) [30,31] in patients treated with abiraterone. A second phase III trial in patients with docetaxel-naive CRPC (COU-AA-302) lead 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 enzautamide 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 filutamide) 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 constitutively activate 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 distinctly different 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 Antonarkis 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

Table
Selected important completed and ongoing phase III trials of secondary hormonal agents in the treatment of prostate cancer

Experimental arm	Control arm	Name of the study	Clinical trials identifier	Clinical state	Prior chemotherapy	Overall survival	Progression-free survival	PSA response
Abiraterone acetate and prednisone [30,31]	Placebo + prednisone	COU-AA-301	NCT00638690	mCRPC	Yes	15.8 mo vs. 11.2 mo ($P < 0.0001$; HR = 0.74)	8.5 mo vs. 6.6 mo ($P < 0.001$; HR = 0.63)[a]	29.5% vs. 5.5% ($P < 0.0001$)
Abiraterone acetate and prednisone [5,32]	Placebo + prednisone	COU-AA-302	NCT00887198	mCRPC	No	34.7 mo vs. 30.3 mo ($P = 0.0033$; HR = 0.81)	16.5 mo vs. 8.3 mo ($P < 0.001$; HR = 0.53)[a]	62 vs. 24 ($P < 0.001$)
[b]Abiraterone acetate and prednisone + ARN-509	Placebo + prednisone		NCT02257736	mCRPC	No	–	–	–
[b]ARN-509	Placebo	SPARTAN	NCT01946204	High-risk, M0 CRPC	Both	–	–	–
[b]ARN-509 + ADT	ADT + placebo		NCT02489318	Low-vol mHSPC	Both	–	–	–
Orteronel and prednisone	Placebo + prednisone	ELM-PC4	NCT01193244	mCRPC	No	31.4 mo vs. 29.5 mo ($P = 0.31$; HR = 0.92)	13.8 mo vs. 8.7 mo ($P < 0.0001$; HR = 0.71)	–
[b]ADT + orteronel	ADT + bicalutamide	SWOG 1216	NCT01809691	mHSPC	No	–	–	–
Enzalutamide [65]	Placebo (±predisone)	AFFIRM	NCT00974311	mCRPC	Yes	18.4 mo vs. 13.6 mo ($P < 0.001$; HR = 0.63)	8.3 mo vs. 2.9[a]–3.0 mo ($P < 0.001$; HR = 0.4[a], HR = 0.25)	54% vs. 2% ($P < 0.001$)
Enzalutamide [4]	Placebo (±predisone)	PREVAIL	NCT01212991	mCRPC	No	At interim analysis, estimated: 32.4 mo vs. 30.2 mo ($P < 0.001$; HR = 0.73)	At 12 mo: 65% vs. 14% ($P < 0.001$; HR = 0.19)[a]	78% vs. 3% ($P < 0.001$)
[b]Enzalutamide + abiraterone acetate + prednisone	Enzalutamide	Alliance A031201	NCT01949337	mCRPC	No	–	–	–
[b]Enzalutamide	ADT	ENZAMET	NCT02446405	New dx mHSPC	No	–	–	–
ODM-201	Placebo	ARAMIS	NCT02200614	M0 CRPC	No	–	–	–
Galeterone	Enzalutamide	ARMOR3-SV	NCT02438007	M1 CRPC (AR-V7 + CTCs)	No	–	–	–

[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 glutamine 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.

References

[1] Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.1, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. International Agency for Research on Cancer. 2014.
[2] National Cancer Institute at National Institute of Health. SEER Stat Fact Sheets: Prostate Cancer. 2015. Accessed 15.05.15.
[3] Friedlander TW, Ryan CJ. Targeting the androgen receptor. Urol Clin North Am 2012;39:453–64.
[4] Beer TM, Armstrong AJ, Rathkopf DE, et al. Enzalutamide in metastatic prostate cancer before chemotherapy. N Engl J Med 2014; 371:424–33.
[5] Ryan CJ, Smith MR, de Bono JS, et al. Abiraterone in metastatic prostate cancer without previous chemotherapy. N Engl J Med 2013; 368:138–48.
[6] Stein MN, Patel N, Bershadskiy A, et al. Androgen synthesis inhibitors in the treatment of castration-resistant prostate cancer. Asian J Androl 2014;16:387–400.
[7] Karantanos T, Evans CP, Tombal B, et al. Understanding the mechanisms of androgen deprivation resistance in prostate cancer at the molecular level. Eur Urol 2015;67:470–9.
[8] Brand LJ, Dehm SM. Androgen receptor gene rearrangements: new perspectives on prostate cancer progression. Curr Drug Targets 2013;14:441–9.
[9] Wang Q, Carroll JS, Brown M. Spatial and temporal recruitment of androgen receptor and its coactivators involves chromosomal looping and polymerase tracking. Mol Cell 2005;19:631–42.
[10] Li J, Al-Azzawi F. Mechanism of androgen receptor action. Maturitas 2009;63:142–8.
[11] Shang Y, Myers M, Brown M. Formation of the androgen receptor transcription complex. Mol Cell 2002;9:601–10.
[12] Koivisto P, Kononen J, Palmberg C, et al. Androgen receptor gene amplification: a possible molecular mechanism for androgen deprivation therapy failure in prostate cancer. Cancer Res 1997;57:314–9.
[13] Holzbeierlein J, Lal P, LaTulippe E, et al. Gene expression analysis of human prostate carcinoma during hormonal therapy identifies androgen-responsive genes and mechanisms of therapy resistance. Am J Pathol 2004;164:217–27.
[14] Bubendorf L, Kononen J, Koivisto P, et al. Survey of gene amplifications during prostate cancer progression by high-throughout fluorescence in situ hybridization on tissue microarrays. Cancer Res 1999;59:803–6.
[15] Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nat Rev Cancer 2001;1:34–45.
[16] Friedlander TW, Roy R, Tomlins SA, et al. Common structural and epigenetic changes in the genome of castration-resistant prostate cancer. Cancer Res 2012;72:616–25.
[17] Stanbrough M, Bubley GJ, Ross K, et al. Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer Res 2006;66:2815–25.
[18] Visakorpi T, Hyytinen E, Koivisto P, et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet 1995;9:401–6.
[19] Taylor BS, Schultz N, Hieronymus H, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11–22.
[20] Locke JA, Guns ES, Lubik AA, et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. Cancer Res 2008;68:6407–15.
[21] Montgomery RB, Mostaghel EA, Vessella R, et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res 2008;68: 4447–54.
[22] Page ST, Lin DW, Mostaghel EA, et al. Persistent intraprostatic androgen concentrations after medical castration in healthy men. J Clin Endocrinol Metab 2006;91:3850–6.

[23] Efstathiou E, Titus M, Tsavachidou D, et al. Effects of abiraterone acetate on androgen signaling in castrate-resistant prostate cancer in bone. J Clin Oncol 2012;30:637–43.
[24] Lamberts SW, Bons EG, Bruining HA, et al. Differential effects of the imidazole derivatives etomidate, ketoconazole and miconazole and of metyrapone on the secretion of cortisol and its precursors by human adrenocortical cells. J Pharmacol Exp Ther 1987;240:259–64.
[25] Small EJ, Halabi S, Dawson NA, et al. Antiandrogen withdrawal alone or in combination with ketoconazole in androgen-independent prostate cancer patients: a phase III trial (CALGB 9583). J Clin Oncol 2004;22:1025–33.
[26] Suzman DL, Antonarakis ES. Castration-resistant prostate cancer: latest evidence and therapeutic implications. Ther Adv Med Oncol 2014;6:167–79.
[27] Attard G, Reid AH, Yap TA, et al. Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. J Clin Oncol 2008;26:4563–71.
[28] Danila DC, Morris MJ, de Bono JS, et al. Phase II multicenter study of abiraterone acetate plus prednisone therapy in patients with docetaxel-treated castration-resistant prostate cancer. J Clin Oncol 2010;28:1496–501.
[29] Ryan CJ, Shah S, Efstathiou E, et al. Phase II study of abiraterone acetate in chemotherapy-naive metastatic castration-resistant prostate cancer displaying bone flare discordant with serologic response. Clin Cancer Res 2011;17:4854–61.
[30] de Bono JS, Logothetis CJ, Molina A, et al. Abiraterone and increased survival in metastatic prostate cancer. N Engl J Med 2011; 364:1995–2005.
[31] Fizazi K, Scher HI, Molina A, et al. Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol 2012;13:983–92.
[32] Ryan CJ, Smith MR, Fizazi K, et al. Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naive men with metastatic castration-resistant prostate cancer (COU-AA-302): final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. Lancet Oncol 2015;16:152–60.
[33] Dreicer R, Agus DB, Macvicar GR, Wang J, Maclean D, Stadler WM. Safety, pharmacokinetics, and efficacy of TAK-700 in metastatic castration-resistant prostrate cancer: a Phase I/II, open-label study. J Clin Oncol 2010;28(15 Suppl):[Abstr 3084].
[34] Dreicer R, Jones R, Oudard S, et al. Results from a phase 3 randomized, double-blind, multicenter, placebo-controlled trial of orteronel (TAK-700) plus prednisone in patients with metastatic castration-resistant prostate cancer (mCRPC) that has progressed during or following docetaxel-based therapy (ELM-PC 5 Trial). Clin Adv Hematol Oncol 2014;12:6–7.
[35] Saad F, Fizazi K, Jinga V, et al. Orteronel plus prednisone in patients with chemotherapy-naive metastatic castration-resistant prostate cancer (ELM-PC 4): a double-blind, multicentre, phase 3, randomised, placebo-controlled trial. Lancet Oncol 2015;16:338–48.
[36] Montgomery RB, Eisenberger MA, Rettig M, et al. Phase I clinical trial of galeterone (TOK-001), a multifunctional antiandrogen and CYP17 inhibitor in castration resistant prostate cancer (CRPC). J Clin Oncol 2012;30 [suppl; abstr 4665].
[37] Montgomery RB, Eisenberger MA, Heath EI, et al. Galeterone in men with CRPC: results in four distinct patient populations from the ARMOR2 study. J Clin Oncol 2014;32(5s) [suppl; abstr 5029].
[38] Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med 2014;371:1028–38.
[39] Eisner JR, Abbott DH, Rafferty SW, et al. VT-464: a novel, selective inhibitor of P450c17(CYP17)-17,20 lyase for castration-refractory prostate cancer (CRPC). J Clin Oncol 30, 2012 ([suppl; abstr e15167]).
[40] Moore WR, Norris JD, Wardell S, et al. Direct effects of the selective CYP17 lyase (L) inhibitor, VT-464, on the androgen receptor (AR) and its oral activity in an F876L tumor mouse xenograft model. J Clin Oncol 2015;33 [suppl 7; abstr 263].
[41] de Bono JS, Pezaro CJ, Gillessen S, et al. The oral CYP17-Lyase (L) inhibitor VT-464 in patients with CRPC. J Clin Oncol 2015;33 [suppl 7; abstr 187].
[42] Azad AA, Volik SV, Wyatt AW, et al. Androgen receptor gene aberrations in circulating cell-free DNA: biomarkers of therapeutic resistance in castration-resistant prostate cancer. Clin Cancer Res 2015;21:2315–24.
[43] Salvi S, Casadio V, Conteduca V, et al. Circulating cell-free AR and CYP17A1 copy number variations may associate with outcome of metastatic castration-resistant prostate cancer patients treated with abiraterone. Br J Cancer 2015;112:1717–24.
[44] Zhao XY, Malloy PJ, Krishnan AV, et al. Glucocorticoids can promote androgen-independent growth of prostate cancer cells through a mutated androgen receptor. Nat Med 2000;6:703–6.
[45] Culig Z, Hobisch A, Cronauer MV, et al. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. Cancer Res 1994;54:5474–8.
[46] Brooke GN, Bevan CL. The role of androgen receptor mutations in prostate cancer progression. Curr Genomics 2009;10:18–25.
[47] Koochekpour S. Androgen receptor signaling and mutations in prostate cancer. Asian J Androl 2010;12:639–57.
[48] Marcelli M, Ittmann M, Mariani S, et al. Androgen receptor mutations in prostate cancer. Cancer Res 2000;60:944–9.
[49] Taplin ME, Bubley GJ, Shuster TD, et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. N Engl J Med 1995;332:1393–8.
[50] Steinkamp MP, O'Mahony OA, Brogley M, et al. Treatment-dependent androgen receptor mutations in prostate cancer exploit multiple mechanisms to evade therapy. Cancer Res 2009;69: 4434–42.
[51] Taplin ME, Bubley GJ, Ko YJ, et al. Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. Cancer Res 1999;59:2511–5.
[52] Ceraline J, Cruchant MD, Erdmann E, et al. Constitutive activation of the androgen receptor by a point mutation in the hinge region: a new mechanism for androgen-independent growth in prostate cancer. Int J Cancer 2004;108:152–7.
[53] Chen G, Wang X, Zhang S, et al. Androgen receptor mutants detected in recurrent prostate cancer exhibit diverse functional characteristics. Prostate 2005;63:395–406.
[54] Bergerat JP, Ceraline J. Pleiotropic functional properties of androgen receptor mutants in prostate cancer. Hum Mutat 2009;30:145–57.
[55] Li W, Cavasotto CN, Cardozo T, et al. Androgen receptor mutations identified in prostate cancer and androgen insensitivity syndrome display aberrant ART-27 coactivator function. Mol Endocrinol 2005;19:2273–82.
[56] Irwin RJ, Prout GR Jr. A new antiprostatic agent for treatment of prostatic carcinoma. Surg Forum 1973;24:536–7.
[57] Airhart RA, Barnett TF, Sullivan JW, et al. Flutamide therapy for carcinoma of the prostate. South Med J 1978;71:798–801.
[58] Sogani PC, Whitmore WF Jr. Experience with flutamide in previously untreated patients with advanced prostatic cancer. J Urol 1979;122:640–3.
[59] Sanford EJ, Bowditch RR, Rohner TJ Jr., et al. Treatment of advanced prostatic cancer with flutamide. Pa Med 1979;82:29–31.
[60] Blackledge GR. Clinical progress with a new antiandrogen, Casodex (bicalutamide). Eur Urol 1996;29(Suppl 2):96–104.
[61] Mahler C, Denis L. Clinical profile of a new non-steroidal antiandrogen. J Steroid Biochem Mol Biol 1990;37:921–4.
[62] Brisset JM, Boccon-Gibod L, Botto H, et al. Anandron (RU 23908) associated to surgical castration in previously untreated stage D

prostate cancer: a multicenter comparative study of two doses of the drug and of a placebo. Prog Clin Biol Res 1987;243A:411–22.
[63] Tran C, Ouk S, Clegg NJ, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. Science 2009;324:787–90.
[64] Higano CS, Beer TM, Taplin ME, et al. Long-term safety and antitumor activity in the phase 1-2 study of enzalutamide in pre- and post-docetaxel castration-resistant prostate cancer. Eur Urol 2015;68:795–801.
[65] Scher HI, Fizazi K, Saad F, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. N Engl J Med 2012;367:1187–97.
[66] Chowdhury S, Heidenreich A, Villers A, et al. Results from TERRAIN: a randomized, double-blind, phase 2 study of enzalutamide vs. bicalutamide in patients with metastatic castration-resistant prostate cancer (mCRPC). J Clin Oncol 33 2015 [suppl; abstr 5049].
[67] Merseburger AS, Haas GP, von Klot CA. An update on enzalutamide in the treatment of prostate cancer. Ther Adv Urol 2015;7:9–21.
[68] Clegg NJ, Wongvipat J, Joseph JD, et al. ARN-509: a novel antiandrogen for prostate cancer treatment. Cancer Res 2012;72:1494–503.
[69] Rathkopf DE, Morris MJ, Fox JJ, et al. Phase I study of ARN-509, a novel antiandrogen, in the treatment of castration-resistant prostate cancer. J Clin Oncol 2013;31:3525–30.
[70] Posadas EM, Chi KN, de Wit R, et al. Phase 1b study of ARN-509 with abiraterone acetate (AA) and prednisone (P) in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC). J Clin Oncol 2015;33 [suppl; abstr 5028].
[71] Fizazi K, Massard C, Bono P, et al. Activity and safety of ODM-201 in patients with progressive metastatic castration-resistant prostate cancer (ARADES): an open-label phase 1 dose-escalation and randomised phase 2 dose expansion trial. Lancet Oncol 2014;15:975–85.
[72] Moilanen A, Riikonen R, Kallio PJ. ODM-201—new generation antiandrogen with excellent antiandrogenic and antitumor activity in nonclinical models of CRPC. Eur J Cancer 2013;49(Suppl 2):[abstr 685].
[73] Lu J, Van der Steen T, Tindall DJ. Are androgen receptor variants a substitute for the full-length receptor? Nat Rev Urol 2015;12:137–44.
[74] Lu C, Luo J. Decoding the androgen receptor splice variants. Transl Androl Urol 2013;2:178–86.
[75] Jenster G, van der Korput HA, van Vroonhoven C, et al. Domains of the human androgen receptor involved in steroid binding, transcriptional activation, and subcellular localization. Mol Endocrinol 1991;5:1396–404.
[76] Dehm SM, Schmidt LJ, Heemers HV, et al. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. Cancer Res 2008;68:5469–77.
[77] Hu R, Dunn TA, Wei S, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. Cancer Res 2009;69:16–22.
[78] Guo Z, Yang X, Sun F, et al. A novel androgen receptor splice variant is up-regulated during prostate cancer progression and promotes androgen depletion-resistant growth. Cancer Res 2009;69:2305–13.
[79] Tepper CG, Boucher DL, Ryan PE, et al. Characterization of a novel androgen receptor mutation in a relapsed CWR22 prostate cancer xenograft and cell line. Cancer Res 2002;62:6606–14.
[80] Hornberg E, Ylitalo EB, Crnalic S, et al. Expression of androgen receptor splice variants in prostate cancer bone metastases is associated with castration-resistance and short survival. PLoS One 2011;6:e19059.
[81] Hu R, Isaacs WB, Luo J. A snapshot of the expression signature of androgen receptor splicing variants and their distinctive transcriptional activities. Prostate 2011;71:1656–67.
[82] Watson PA, Chen YF, Balbas MD, et al. Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor. Proc Natl Acad Sci U S A 2010;107:16759–65.
[83] Sun F, Indran IR, Zhang ZW, et al. A novel prostate cancer therapeutic strategy using icaritin activated arylhydrocarbon-receptor to co-target androgen receptor and its splice variants. Carcinogenesis 2015;36:757–68.
[84] Cao B, Qi Y, Zhang G, et al. Androgen receptor splice variants activating the full-length receptor in mediating resistance to androgen-directed therapy. Oncotarget 2014;5:1646–56.
[85] Antonarakis ES, Lu C, Luber B, et al. Androgen receptor splice variant 7 and efficacy of taxane chemotherapy in patients with metastatic castration-resistant prostate cancer. JAMA Oncology 2015;1:582–91.
[86] Sadar MD, Williams DE, Mawji NR, et al. Sintokamides A to E, chlorinated peptides from the sponge Dysidea sp. that inhibit transactivation of the N-terminus of the androgen receptor in prostate cancer cells. Org Lett 2008;10:4947–50.
[87] Andersen RJ, Mawji NR, Wang J, et al. Regression of castrate-recurrent prostate cancer by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor. Cancer Cell 2010;17:535–46.
[88] Sadar MD. Small molecule inhibitors targeting the “achilles’ heel” of androgen receptor activity. Cancer Res 2011;71:1208–13.
[89] Liu C, Lou W, Zhu Y, et al. Niclosamide inhibits androgen receptor variants expression and overcomes enzalutamide resistance in castration-resistant prostate cancer. Clin Cancer Res 2014;20:3198–210.
[90] Nelson KA, Witte JS. Androgen receptor CAG repeats and prostate cancer. Am J Epidemiol 2002;155:883–90.
[91] Feldman D. Androgen and vitamin D receptor gene polymorphisms: the long and short of prostate cancer risk. J Natl Cancer Inst 1997;89:109–11.
[92] Zeegers MP, Kiemeney LA, Nieder AM, et al. How strong is the association between CAG and GGN repeat length polymorphisms in the androgen receptor gene and prostate cancer risk? Cancer Epidemiol Biomarkers Prev 2004;13:1765–71.
[93] Price DK, Chau CH, Till C, et al. Androgen receptor CAG repeat length and association with prostate cancer risk: results from the prostate cancer prevention trial. J Urol 2010;184:2297–302.
[94] Figg WD, Chau CH, Price DK, et al. Androgen receptor CAG repeat length and TMPRSS2: ETS prostate cancer risk: results From the Prostate Cancer Prevention Trial. Urology 2014;84:127–31.
[95] Mao X, Li J, Xu X, et al. Involvement of different mechanisms for the association of CAG repeat length polymorphism in androgen receptor gene with prostate cancer. Am J Cancer Res 2014;4:886–96.
[96] Suzuki H, Ueda T, Ichikawa T, et al. Androgen receptor involvement in the progression of prostate cancer. Endocr Relat Cancer 2003;10:209–16.
[97] Klotz L, Correia A, Zhang W. The relationship between the androgen receptor CAG repeat polymorphism length and the response to intermittent androgen suppression therapy for advanced prostate cancer. Prostate Cancer Prostatic Dis 2005;8:179–83.
[98] Misra D, Xie W, Regan MM, et al. Germline CAG repeat length of the androgen receptor and time to progression in patients with prostate cancer treated with androgen deprivation therapy. BJU Int 2011;108:1086–91.
[99] Shimbo M, Suzuki H, Kamiya N, et al. CAG polymorphic repeat length in androgen receptor gene combined with pretreatment serum testosterone level as prognostic factor in patients with metastatic prostate cancer. Eur Urol 2005;47:557–63.
[100] Southwell J, Chowdhury SF, Gottlieb B, et al. An investigation into CAG repeat length variation and N/C terminal interactions in the

T877A mutant androgen receptor found in prostate cancer. J Steroid Biochem Mol Biol 2008;111:138–46.

[101] Chen C, Lamharzi N, Weiss NS, et al. Androgen receptor polymorphisms and the incidence of prostate cancer. Cancer Epidemiol Biomarkers Prev 2002;11:1033–40.

[102] Stanford JL, Just JJ, Gibbs M, et al. Polymorphic repeats in the androgen receptor gene: molecular markers of prostate cancer risk. Cancer Res 1997;57:1194–8.

[103] Beltran H, Prandi D, Mosquera JM, et al. Defining a molecular subclass of treatment resistant prostate cancer. J Clin Oncol 2015;33 [suppl; abstr 5004].

[104] Aggarwal RR, Thomas G, Youngren J, et al. Androgen receptor (AR) amplification in patients (pts) with metastatic castration resistant prostate cancer (mCRPC) resistant to abiraterone (Abi) and enzalutamide (Enz): Preliminary results from the SU2C/PCF/AACR West Coast Prostate Cancer Dream Team (WCDT). J Clin Oncol 2015;33 [suppl; abstr 5068].