Androgen receptor signaling in castration-resistant prostate cancer: a lesson in persistence

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Abstract

The androgen receptor (AR) signaling axis drives all stages of prostate cancer, including the lethal, drug-resistant form of the disease termed castration-resistant prostate cancer (CRPC), which arises after failure of androgen deprivation therapy (ADT). Persistent AR activity in spite of ADT and the second-generation AR-targeting agents enzalutamide and abiraterone is achieved in many cases by direct alterations to the AR signaling axis. Herein, we provide a detailed description of how such alterations contribute to the development and progression of CRPC. Aspects of this broad and ever-evolving field specifically addressed in this review include: the etiology and significance of increased AR expression; the frequency and role of gain-of-function mutations in the *AR* gene; the function of constitutively active, truncated forms of the AR termed AR variants and the clinical relevance of alterations to the activity and expression of AR coregulators. Additionally, we examine the novel therapeutic strategies to inhibit these classes of therapy resistance mechanisms, with an emphasis on emerging agents that act in a manner distinct from the current ligand-centric approaches. Throughout, we discuss how the central role of AR in prostate cancer and the constant evolution of the AR signaling axis during disease progression represent archetypes of two key concepts in oncology, oncogene addiction and therapy-mediated selection pressure.

Key Words
- androgen receptor
- prostate
- endocrine therapy resistance
- hormone structure/function

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Introduction

Prostate cancer (PCa) is the second most commonly diagnosed cancer and a leading cause of cancer-related death in men worldwide (Ferlay *et al.* 2015). Indeed, in the USA alone, 180,890 new PCa cases are expected to be diagnosed in 2016 (Siegel *et al.* 2016). Given its critical role in the normal prostate, it is perhaps not surprising that the AR signaling axis is critical for PCa genesis and all subsequent phases of disease progression. This biology underpins the use of androgen deprivation therapy (ADT), a term used to describe hormonal manipulations aimed at reducing androgen levels and/or blocking AR activity, as the mainstay treatment for locally advanced or metastatic disease. Although ADT is initially successful in almost all men, development of resistance is inevitable, normally occurring within a period of 2–3 years. The resultant form of the disease, termed as castration-resistant prostate cancer (CRPC), is incurable and lethal (Scher *et al.* 2004, Thoreson *et al.* 2014).

Given the efficacy of ADT in suppressing circulating testosterone levels, it was believed that CRPC was

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an AR-independent pathology, leading to its early designations as 'hormone-refractory' or 'androgen-independent' PCa. However, a substantial body of work over the past ~20 years has demonstrated that the majority of CRPC cases remain dependent on the AR signaling axis. One of the first clues to this concept was the observation of *AR* gene amplification in over 30% CRPC of patients after hormonal therapy (Visakorpi *et al*. 1995). Another early indicator of the relevance of AR in CRPC came from the detection of AR gain-of-function point mutations that were associated with rapid failure of endocrine therapy (Tilley *et al*. 1996). Subsequently, an elegant study from the Sawyers group showed that reactivation of AR signaling is sufficient and necessary to trigger the CRPC phenotype (Chen *et al*. 2004). These revelations led to the development of new and more efficacious AR-targeting agents, the AR antagonist enzalutamide and the androgen synthesis inhibitor abiraterone, which provided survival benefits for men with CRPC and definitively proved an ongoing dependence on AR signaling in this disease setting (de Bono *et al*. 2011, Scher *et al*. 2012, Ryan *et al*. 2013, Beer *et al*. 2014).

Although this review is focused on oncogenic functions of AR in the prostate, the requirement for AR signaling in normal physiology bears consideration, especially given the widespread use of ADT. AR is almost ubiquitously expressed in the tissues of both males and females, enabling it to mediate a plethora of vital regulatory functions (Lee & Chang 2003, De Gendt & Verhoeven 2012). For example, AR is important for the acquisition and maintenance of bone mass by suppressing and stimulating the apoptosis of osteoblasts and osteoclasts, respectively (Mohamad *et al*. 2016). In the muscle, the AR signaling axis is critical for muscle growth, development and regeneration (Velders & Diel 2013). AR also influences brain biology and function, and its role in promoting neuron health and growth possibly underlies the link between ADT and dementia (Nead *et al*. 2016). In females, among other functions, AR is required for normal fertility (De Gendt & Verhoeven 2012) and plays an important anti-proliferative role in the breast (Hickey *et al*. 2012). The critical reproductive and non-reproductive functions of AR outlined previously are manifested by the adverse side effects associated with ADT, which include sexual dysfunction, decreased lean body mass and strength, osteoporosis, increased cardiovascular disease and cognitive decline (for a detailed review, see Ahmadi & Daneshmand 2013).

Mechanisms of persistent AR signaling activity in CRPC

As the major driver of CRPC, there is a critical need to better understand how AR signaling persists in a castration environment. The advent of the omics era, and resultant integrative genomic analyses of metastatic samples, has further highlighted the extent and frequency of AR pathway alterations in CRPC that may contribute to inappropriate activation or reactivation of this pathway (Grasso *et al*. 2012, Beltran *et al*. 2013, Azad *et al*. 2015, Robinson *et al*. 2015, Lallous *et al*. 2016). Although these alterations have been the subject of other recent excellent articles (for example Penning 2014, Joshi *et al*. 2015, Wyatt & Gleave 2015), this review will specifically focus on ADT-resistance mechanisms driven by direct structural changes to the AR and altered interplay between the AR and its coregulators. Finally, we will summarize the recent progress into the development of novel therapeutic strategies to target these specific resistance mechanisms.

ADT-resistance mechanisms driven by direct structural changes to the AR

AR overexpression Increased expression of AR is one of the most frequent alterations observed in CRPC (Grasso *et al*. 2012, Beltran *et al*. 2013, Robinson *et al*. 2015) and has been consistently associated with the development of resistance to anti-androgens (Chen *et al*. 2004, Kawata *et al*. 2010). Elevated AR hypersensitizes cancer cells to castrate levels of androgens (Visakorpi *et al*. 1995, Koivisto *et al*. 1997, Kawata *et al*. 2010), mediates antagonist–agonist switching (Chen *et al*. 2004) and can promote resistance to AR-targeting agents (Carreira *et al*. 2014).

Increased AR expression in CRPC is often mediated by *AR* gene amplification. Pre-treated CRPC tumors exhibit *AR* amplification rates of 17–57%, depending on the therapy (Grasso *et al*. 2012, Beltran *et al*. 2013, Robinson *et al*. 2015, Kumar *et al*. 2016). This is in contrast to treatment-naïve tumors, in which copy number gain is rarely detected (Visakorpi *et al*. 1995, Koivisto *et al*. 1997, Carreira *et al*. 2014, Cancer Genome Atlas Research 2015, Kumar *et al*. 2016). *AR* gene amplification has also been detected in circulating tumor cells (CTCs) and cell-free circulating tumor DNA (ctDNA) from patients with CRPC (Antonarakis *et al*. 2014, Carreira *et al*. 2014, Azad *et al*. 2015).

Although *AR* amplification is frequent in CRPC and an important driver of therapy resistance, AR levels can be increased irrespective of gene copy number status

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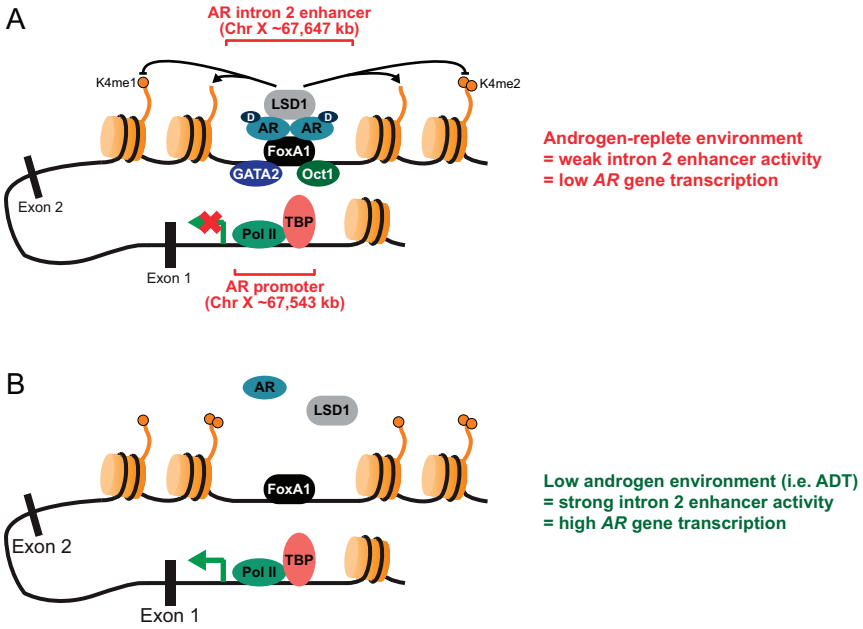

Figure 1
Model for negative feedback loop mediated by AR (AR autoregulation). (A) Recruitment of ligand-bound AR by FoxA1 to an enhancer in intron 2 of the *AR* gene results in lysine-specific histone demethylase 1 (LSD1)-mediated demethylation of mono- and di-methylated lysine 4 (K4me1 and K4me2, respectively) on histone H3 (LSD1 also demethylates K9 at this loci). Formation of this repressive chromatin environment at the intron 2 enhancer, which also involves the transcription factors GATA2 and Oct1, directly suppresses *AR* gene transcription via chromatin looping back to the promoter. Chromosome coordinates correspond to hg38 assembly. (B) In a low androgen environment, such as occurs during androgen deprivation therapy (ADT), intron 2 enhancer activity is no longer suppressed by ligand-bound AR and LSD1, resulting in increased *AR* gene transcription. D, DHT; Me, methyl; Me2, dimethyl; Pol II, RNA polymerase II; TBP, TATA-binding protein. This model is based primarily on data from Cai *et al.* (2011).

through transcriptional upregulation. Importantly, a direct mechanism underlying ADT-driven upregulation of the *AR* gene has been elucidated: liganded AR negatively regulates its own expression by binding to a site in the second intron of the *AR* gene, and this repression is relieved by AR-targeted therapy (Cai *et al.* 2011) (Fig. 1). More recently, Spratt and coworkers demonstrated that radiotherapy can also induce *AR* gene transcription, a response correlated with increased cancer cell survival *in vitro* and decreased time to disease progression *in vivo* (Spratt *et al.* 2015).

***AR* gene mutations** In CRPC, *AR* mutations have been reported to occur at a frequency of 5–30% in pre-treated tumors (Taplin *et al.* 1999, Wallen *et al.* 1999, Grasso *et al.* 2012, Beltran *et al.* 2013, Robinson *et al.* 2015, Kumar *et al.* 2016), CTCs (Jiang *et al.* 2010) and ctDNA (Azad *et al.* 2015, Lallous *et al.* 2016, Wyatt *et al.* 2016). Although infrequent, *AR* mutations have also been detected in primary PCa prior to ADT or arising during treatment, and there is evidence that therapy-mediated selection of such mutations can underlie resistance in some patients (Tilley *et al.* 1996, Taplin *et al.* 1999, Thompson *et al.* 2003, Steinkamp *et al.* 2009, Carreira *et al.* 2014, Cancer Genome Atlas Research 2015, Chen *et al.* 2015). Most AR mutations cluster in domains responsible for ligand-binding (the AR ligand-binding domain, referred to as AR-LBD) or transactivation activity (the AR N-terminal domain, referred to as AR-NTD).

Mutations detected recurrently (>1 sample) in PCa are listed in Table 1, whereas a comprehensive list of PCa-associated *AR* mutations and their proposed functions is presented in Supplementary Table 1, see section on supplementary data given at the end of this article. Most mutations identified in CRPC cluster in the AR-LBD. These alterations have been reported to facilitate AR signaling in CRPC by conferring: (i) ligand promiscuity, thereby allowing AR to be activated even in the presence of low/absent levels of androgens and (ii) agonist properties to AR antagonists (Supplementary Table 1). Recurrently occurring LBD missense mutations in CRPC are L702H, W742C, H875Y and T878A (Grasso *et al.* 2012, Robinson *et al.* 2015, Lallous *et al.* 2016). With the exception of W742C, these AR mutants are represented in commonly used PCa cell lines (T878A in LNCaP, C42B and MDA-PCa-2B; L702H in MDA-MB-2B and H875Y in 22Rv1 and CWR-R1), which has facilitated our understanding of their function. The T878A mutant, first identified in the LNCaP cell line (Veldscholte *et al.* 1990), is the archetypal promiscuous receptor, being activated by progesterone, estrogen and glucocorticoids (Veldscholte *et al.* 1990, Berrevoets *et al.* 1993, Suzuki *et al.* 1996, Culig *et al.* 1999, Zhao *et al.* 2000, Steketee *et al.* 2002, Chen *et al.* 2015, Lallous *et al.* 2016). H875Y and L702H also broaden ligand specificity by enabling AR activation by glucocorticoids (Suzuki *et al.* 1993, Taplin *et al.* 1995, Zhao *et al.* 2000, Steketee *et al.* 2002). Importantly, mutations conferring ligand promiscuity not only can facilitate persistent AR signaling in the castrate environment but

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Table 1 Recurrent prostate cancer-associated AR missense mutations.

				Mutations per sample									
Treatment	**Stage of disease**	***Np***	***Ns***	T878A	H875Y	L702H	ΔQ86	W742C	Q58L	M896V	E213E	Q868*	T878S	T229C
CRPC	Metastatic	150	150	7	4	7		2	1				1	
		63	176		6	1		1						
		62	65	5	9					3				
		51	70		1	3		2						
		50		3	2	1								
		48	48	1				1						
		33	33	6									1	
		24		6										
		22	22	3										
		10	10	1	1								1	
		8	8				5		4		1	1		2
		8	8	1										
		7	7											
	Localized	8	8			1								
Hormone-naïve	Metastatic	38	38									1		
		3	3				2		1			1		
	Localized	499							1					
		26	26											
		23												
Non-specified	Metastatic	37		2	1			1						
		1	1											
	Localized	67	67							1	2			

AR, androgen receptor; CRPC, castration-resistant prostate cancer; *Np*, number of patients enrolled; *Ns*, number of samples.

also can result in qualitative changes to the receptor. For example, it has been shown that non-canonical ligands activate specific subsets of genes with relevance to prostate cell proliferation (Brooke *et al*. 2008, Zaman *et al*. 2014). Moreover, proteomic and targeted analysis of AR-associated factors revealed that H875Y, T878A and T878S recruit distinct coactivators in response to different ligands (Brooke *et al*. 2008, Zaman *et al*. 2014). The T878A mutation also facilitates the interaction with the coactivator steroid receptor coactivator (SRC) 1 and weakens the interaction with the co-repressor NCOR1 (Berrevoets *et al*. 2004).

The other major phenotypic output of common LBD mutations is antagonist–agonist switching (Supplementary Table 1), which likely explains the withdrawal syndrome observed after cessation of first-generation antagonists seen in 15–30% of patients (Small *et al*. 2004). T878A confers agonist properties to flutamide and nilutamide, H875Y to nilutamide and W742C/L to bicalutamide (Veldscholte *et al*. 1990, Suzuki *et al*. 1996, Tan *et al*. 1997, Hara *et al*. 2003, Azad *et al*. 2015, O'Neill *et al*. 2015, Lallous *et al*. 2016). Interestingly, O'Neill and coworkers recently demonstrated that T878A inhibited bicalutamide-activated W742L (O'Neill *et al*. 2015) in what may represent the first evidence of antagonism arising from the heterodimerization of distinct AR mutants. Another mutation that can confer antagonist–agonist switching is F877L, which was found to be activated by enzalutamide in cell lines (Balbas *et al*. 2013, Joseph *et al*. 2013, Korpal *et al*. 2013). Although this alteration was identified in circulating DNA from a small proportion of CRPC patients who were progressing on enzalutamide or ARN-509 (Joseph *et al*. 2013, Azad *et al*. 2015), F877L was not detected in a recent study of 150 CRPC metastases, 48% of which were pre-treated with enzalutamide, suggesting that it may not be a key resistance mechanism (Robinson *et al*. 2015).

Mutations in the AR-NTD, which account for about a third of all mutations described in AR (Steinkamp *et al*. 2009) (Supplementary Table 1), usually cause alterations that facilitate AR transactivation activity, such as altered recruitment of coactivators and other components of the transcriptional machinery, increased N/C interaction, increased response to 5α-dihydrotestosterone (DHT) activation and enhanced protein stability and nuclear retention (Tilley *et al*. 1996, Buchanan *et al*. 2004, Chen *et al*. 2005, Callewaert *et al*. 2006, Steinkamp *et al*. 2009).

Altogether, there is overwhelming evidence that *AR* mutation represents a key mechanism underlying persistent AR signaling in CRPC. Therefore, monitoring

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A253V	W435L	T440P	G456S	G457D	R485C	T498I	V509L	C620Y	V716M	V731M	R787*	S889G	Study
													Robinson *et al*. (2015)
													Kumar *et al*. (2016)
												2	Lallous *et al*. (2016)
													Beltran *et al*. (2016)
													Grasso *et al*. (2012)
													Taplin *et al*. (2003)
													Taplin *et al*. (1999)
													Gaddipati *et al*. (1994)
													Suzuki *et al*. (1996)
													Taplin *et al*. (1995)
2	2	2	2	1	1	1	2		1		1		Steinkamp *et al*. (2009)
													Suzuki *et al*. (1993)
									1				Culig *et al*. (1993)
													Suzuki *et al*. (1993)
								1					Marcelli *et al*. (2000)
				1	1	1					1		Steinkamp *et al*. (2009)
													Cancer Genome Atlas Research Network (2015)
										1			Newmark *et al*. (1992)
													Elo *et al*. (1995)
													Taylor *et al*. (2010)
								1					Nazareth *et al*. (1999)
										1			Sanchez *et al*. (2006)

the emergence of *AR* mutations in real time could guide therapy selection, especially in light of the observations that individual mutations do not appear to confer resistance to all AR antagonists (Lallous *et al*. 2016). In this respect, the emerging capacity to rapidly characterize ctDNA represents an exciting prospect (Carreira *et al*. 2014, Azad *et al*. 2015). Additionally, a better understanding of the molecular function of mutant ARs is required to achieve maximal patient benefit. The field has traditionally been limited by a scarcity of CRPC models for studying AR mutant function, but new models derived from patient metastases and CTCs, such as patient-derived xenografts and organoids, are emerging (Gao *et al*. 2014, Lin *et al*. 2014, Alsop *et al*. 2016). As these models encompass the diversity of disease and AR alterations, they are likely to have a profound impact on our knowledge of aberrant AR signaling.

AR splice variants The addiction of prostate cancer cells to AR, manifested by extreme pressure to maintain AR expression and activity, is also associated with the emergence of truncated forms of the receptor. Many of these AR variants (AR-Vs) lack all or part of the transcript encoding the AR-LBD and can be divided into two main classes: those that incorporate a cryptic exon or those arising from exon skipping. Rapid, reversible induction of AR-V expression can be achieved by alternative splicing (Watson *et al*. 2010, Hu *et al*. 2012, Gillis *et al*. 2013, Liu *et al*. 2013, Yu *et al*. 2014*b*), whereas genomic rearrangements within the *AR* gene can mediate a fully androgen-independent phenotype through a mechanism of switching AR expression from full-length AR to ARv567es (Nyquist *et al*. 2013).

AR-V mRNAs have been identified in prostate cancer cell lines, xenografts, mouse models and, most importantly, patient specimens (Dehm *et al*. 2008, Guo *et al*. 2009, Hu *et al*. 2009, Sun *et al*. 2010, Watson *et al*. 2010, Hornberg *et al*. 2011, McGrath *et al*. 2013, Robinson *et al*. 2015) (Table 2). The most recent genomic data, based on the detection of unique exon junctions in RNAseq data, estimates that at least 12 distinct *AR-V* mRNA species are detectable in primary PCa (Cancer Genome Atlas Research 2015) and 23 in mCRPC (Robinson *et al*. 2015) (for a comprehensive summary of the AR splicing landscape, Supplementary Table 2). The best characterized AR-Vs are AR-V7 and ARv567es, which are discussed in more detail below. The structural properties of AR-Vs, namely, the lack of an intact LBD, theoretically confer ligand-independent activity. However, this concept has only been proven for a subset of AR-Vs (Supplementary Table 2).

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Table 2 The landscape of AR variants in clinical prostate cancer.

	Name	Exon structure	Distinguishing exon junction	Transcriptional activity	Detected in disease stage
Inclusion of non-canonical exon	AR-V1	1/2/3/CE1	3/CE1	Depends on cell context	Benign, hormone-naïve and CRPC; primary and metastases (higher in CRPC and metastases)
	AR-V3	1/2/CE4	2/CE4	Constitutive	Increased levels castrated vs non-treated xenografts; peritumoral, benign, primary tumor and mCRPC
	AR-V5	1/2/3/CE2	3/CE2	Unknown	Primary tumor and mCRPC
	AR-V6	1/2/3/CE2′	3/CE2′	Unknown	Primary tumor and mCRPC
	AR-V7	1/2/3/CE3	3/CE3	Constitutive	Benign, hormone-naïve and CRPC; primary and metastases (higher in CRPC and metastases)
	AR-V8	1/2/3/I3	3/I3	Unknown	Primary tumor and mCRPC
	AR-V9	1/2/3/CE5	3/CE5	Depends on cell context	Benign, hormone-naïve and CRPC; primary and metastases (higher in CRPC and metastases)
	AR8	1/I2/3	I2/3	Increases phosphorylation of AR-FL	Benign, malignant
	AR23	1/2/I2/3/4/5/6/7/8	2/I2, I2/3	Ligand-stimulated	Peritumoral, primary tumor and mCRPC
Exon skipping	ARv567es	1/2/3/4/8	4/8	Constitutive	Benign, hormone-naïve and CRPC (higher in CRPC xenografts and bone metastases over hormone-naïve)
	AR-V13	1/2/3/4/5/6/9	6/9	Inactive	Primary tumor and mCRPC (higher in mCRPC)
	AR-V14	1/2/3/4/5/6/7/9	7/9	Inactive	CRPC
	ARv5es	Unknown	4/6	Unknown	Primary tumor and mCRPC (higher in mCRPC)
	ARv56es	Unknown	4/7	Unknown	mCRPC
	ARv7es	Unknown	6/8	Unknown	Primary tumor and mCRPC (higher in mCRPC)
	ARv4es	Unknown	3/5	Unknown	Primary tumor and mCRPC (higher in mCRPC)
	ARv6es	Unknown	5/7	Unknown	Primary tumor and mCRPC
	Exon 1-CE4	Unknown	1/CE4	Unknown	mCRPC
	ARv2es	Unknown	1/3	Unknown	Peritumoral, primary tumor and mCRPC
	ARv3es	Unknown	2/4	Unknown	Peritumoral, primary tumor and mCRPC
	ARv34es	Unknown	2/5	Unknown	Primary tumor and mCRPC
Promoter usage	AR-45	1b/2/3/4/5/6/7/8	1b/2	Depends on cell context	Peritumoral, benign, primary tumor and mCRPC

AR-V, androgen receptor variant; CE, cryptic exon; CRPC, castration-resistant prostate cancer; I, intron; mCRPC, metastatic CRPC.

Expression of AR-Vs in clinical samples has provided evidence for clinically relevant functions. For example, AR-V7 is elevated in castrate-resistant vs hormone-responsive tumor tissues (Guo *et al*. 2009, Hu *et al*. 2009), in CRPC vs benign or localized PCa (McGrath *et al*. 2013) and in CRPC bone metastases compared to benign tissues, primary tumors or hormone-naïve bone metastases (Hornberg *et al*. 2011). Although therapy-driven increases in AR-V7 expression could be due to ADT-mediated alterations to AR splicing factors, RNA-binding proteins or miRNAs (Liu *et al*. 2014*b*, Shi *et al*. 2015, Stockley *et al*. 2015), it must be noted that ligand depletion can result in increased expression of the *AR* gene due to transcriptional autoregulation (described above) (Cai *et al*. 2011). As total *AR* gene expression is highly correlated with alternative splicing (Liu *et al*. 2014*b*, Hickey *et al*. 2015), the association between *AR-V* mRNA and therapy does not necessarily equate to a biologically relevant function in terms of disease progression.

Although the associations between AR-Vs (mRNA and protein) with clinical parameters and drug resistance are compelling, the relevance of these findings has been called into question by the observation that *AR-V* expression at the mRNA level may only be approximately 0.03–7% of the full-length transcript in CRPC metastases (Hornberg *et al*. 2011, Robinson *et al*. 2015). However, we believe that this is a poor reason to discount the biological relevance of AR-Vs. First, as full-length AR (AR-FL) expression is increased by approximately an order of magnitude in CRPC compared to hormone-sensitive disease (Hu *et al*. 2009), the aforementioned ratio is misleading in terms of absolute expression. Second, one study reported that AR-Vs and AR-FL proteins could exist at comparable levels in CRPC bone metastases even though much lower levels of AR-V mRNA were detected, suggesting that AR-Vs could

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be more stable than AR-FL (Hornberg *et al.* 2011). More broadly, direct comparison between the ligand-dependent AR-FL and ligand-independent truncated AR-Vs are problematic as the latter may require much lower protein copies to achieve robust activity. Finally, a recent study provided evidence for the concept that the low levels of AR-V expression evident after ADT allowed tumor cells to retain basal AR activity and thereby survive until more potent AR-activating mechanisms emerged (Yu *et al.* 2014*b*).

Controversy surrounding the biological relevance of AR-Vs, at least in part arising from their low expression in clinical samples, has been compounded by the lack of AR-V-specific antibodies, which means that the existence of endogenous protein products for most *AR-V* transcripts has not been verified in any biological system. AR-V7 is the exception: using an antibody specific to this variant, two groups have demonstrated that it is associated with therapy resistance and poor outcome (Efstathiou *et al.* 2015, Welti *et al.* 2016). Indeed, the recent analytical and clinical validation of an AR-V7 IHC method (Welti *et al.* 2016) demonstrated that it is associated with overall survival in men with CRPC, whereas AR N-terminal domain staining is not. The recent development of a specific and sensitive antibody for ARv567es (Prof. Stephen Plymate, personal communication) is also a significant step forward in the field.

Although the role of AR-Vs as a key driver of CRPC remains equivocal, recent findings have suggested that AR-V7 could have value as a predictive biomarker in CRPC. In a seminal study, Antonarakis and coworkers showed that men with detectable expression of *AR-V7* mRNA in CTCs had reduced response rates to enzalutamide and abiraterone (Antonarakis *et al.* 2014). More recent work has revealed that *AR-V7* transcript is also detectable in whole blood (Liu *et al.* 2016) and predicts poor response to abiraterone (Todenhofer *et al.* 2016). The predictive value of AR-V7 is not limited to transcript expression as a recently developed CTC-based immunofluorescence assay demonstrated that nuclear expression of AR-V7 predicts worse survival after treatment with AR-targeted therapies (Scher *et al.* 2016). Importantly, as the efficacy of chemotherapy appears to be independent of AR-V7 expression in CTCs and tissues, AR-V7 can potentially be used to guide patient therapy (Antonarakis *et al.* 2015, Onstenk *et al.* 2015, Scher *et al.* 2016), an idea that is now being tested in multiple clinical trials (e.g., NCT02429193, NCT02269982, NCT02853097 and NCT02491411). Although the outcomes of these trials are eagerly awaited, it must be noted that exceptions have already been reported: a recent study found that AR-V7 status in CTCs cannot entirely predict non-response to abiraterone or enzalutamide (Bernemann *et al.* 2016). Given that effective alternative treatment options are still somewhat limited, this latter study raises a note of caution for clinicians.

A key question in the field has been whether AR-Vs regulate a classical AR-driven transcriptional program or whether they have distinct gene targets, with evidence for both concepts being reported (Guo *et al.* 2009, Hu *et al.* 2009, 2012, Sun *et al.* 2010, Chan *et al.* 2012, 2015, Tsai *et al.* 2012, Cao *et al.* 2014, Lu *et al.* 2015). Recently, work from Dehm's group has provided a possible explanation for these discrepant findings by identifying proliferative vs anti-proliferative AR gene signatures mediated by differences in DHT dose or AR expression, with the former mitotic gene signature resembling that driven by AR-Vs (Li *et al.* 2013). Multiple other lines of evidence support the idea that AR-Vs are likely to largely recapitulate AR-FL function. First, a recent study from our group found that AR-FL and ARv567es cistromes were highly concordant, with both exhibiting a preference for canonical inverted repeat androgen response elements (Chan *et al.* 2015). Second, dimerization of AR-Vs is required for their transcriptional activity and utilizes the same interaction surface as AR-FL (Chan *et al.* 2015, Xu *et al.* 2015). Finally, transcriptional repression of AR-Vs by binding of the transcription factor FOXO1 to transcription activation unit 5 (TAU5) in the NTD revealed that, like AR-FL, they require this domain for transcriptional activity.

Although we favor the idea that AR-Vs generally regulate a classical androgen transcriptome for the reasons described previously, we note that most studies to date have assessed AR-V activity in isolation. The finding that AR-Vs can heterodimerize with AR-FL or with other AR-Vs (Sun *et al.* 2010, Cao *et al.* 2014, Xu *et al.* 2015) raises the possibility that the nature and relative expression of different AR-Vs and AR-FL in single cells could influence distinct transcriptional outputs.

AR coregulator alterations in CRPC

In the non-pathological state, the interplay of AR with its coregulators within the nucleus is tightly controlled and is essential for the regulation of genomic, ligand-activated functions (Mestayer *et al.* 2003, Xu *et al.* 2009*a*). In the malignant and castration-resistant states, deregulation of this interplay is common and frequently manifested by increased expression and activity of AR coactivators with a concomitant inhibition or loss of AR corepressors

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(Supplementary Table 3). One class of coregulators that plays a key role in facilitating aberrant AR signaling in CRPC is the steroid receptor coactivators (SRC-1, SRC-2 and SRC-3). The expression of all 3 SRCs is frequently elevated in primary disease and associated with disease progression and poor outcome (Fujimoto *et al*. 2001, Gregory *et al*. 2001, Linja *et al*. 2004, Agoulnik *et al*. 2006, Taylor *et al*. 2010). Moreover, aberrant upregulation of the SRCs is even more pronounced in CRPC (Taylor *et al*. 2010, Grasso *et al*. 2012, Cancer Genome Atlas Research 2015, Robinson *et al*. 2015, Beltran *et al*. 2016, Kumar *et al*. 2016). Mechanistically, increased SRC expression enhances AR signaling in low androgen settings and also potentiates alternative ligand usage (Agoulnik & Weigel 2009, Foley & Mitsiades 2016). The clinical relevance of these factors is perhaps best emphasized by the finding that constitutive overexpression of SRC-2 in the mouse prostate epithelium was sufficient for the development of prostate adenocarcinoma in mice, whereas *SRC-2* depletion prevented CRPC development in PTEN-deficient mice (Qin *et al*. 2014).

Another type of AR regulatory molecule is the 'pioneer' factor, with the archetypal example being Forkhead Box A1 (FOXA1). FOXA1 is not a classic transcriptional coactivator but rather serves to open sites of condensed chromatin to facilitate – or 'pioneer' – AR binding, resulting in enhanced transcriptional activity (Wang *et al*. 2009). Amplification and overexpression of *FOXA1* have been detected in primary tumors but is more common in metastatic CRPC, highlighting its role in persistent AR signaling in the castrate state (Jain *et al*. 2011, Grasso *et al*. 2012, Cancer Genome Atlas Research 2015, Robinson *et al*. 2015, Kumar *et al*. 2016). Overexpression of FOXA1 has been associated with poor outcome and AR expression (Jain *et al*. 2011, Sahu *et al*. 2011, Gerhardt *et al*. 2012, Robinson *et al*. 2014). Mechanistically, high levels of FOXA1 in tumor cells may enhance AR:chromatin interactions when androgen levels are low and also enable binding of AR to non-canonical sites, both of which can drive a CRPC gene expression program (Wang *et al*. 2009, Robinson *et al*. 2014). Interestingly, the converse also appears to be true: a number of studies have found that loss of FOXA1 can enable androgen-independent AR chromatin binding at non-canonical sites throughout the genome, and this cistromic reprogramming has been associated with enhanced AR signaling in CRPC (Sahu *et al*. 2011, Wang *et al*. 2011, Jin *et al*. 2014). Thus, maintaining stable FOXA1 expression and activity appears to be an important requirement for the healthy prostate, with deregulation in either direction being potentially oncogenic (see Yang & Yu 2015 for a recent review on this topic). This phenomenon is supported by clinical observations: although *FOXA1* amplification is relatively common in CRPC, loss-of-function *FOXA1* mutations have also been found at a high incidence (Barbieri *et al*. 2012, Grasso *et al*. 2012, Gao *et al*. 2014, Robinson *et al*. 2015, Kumar *et al*. 2016).

GATA2 is another AR pioneer factor with a multifaceted role in the AR signaling axis: it promotes AR (and AR-V) expression, is required for AR transcriptional activity and enhances AR:chromatin associations (Bohm *et al*. 2009, Seo *et al*. 2013, He *et al*. 2014). Given this triumvirate of oncogenic functions, it is not surprising that GATA2 is overexpressed in CRPC and associated with poor outcome (Chiang *et al*. 2014, He *et al*. 2014). GATA2 also has an intimate, bidirectional relationship with FOXA1, with important implications for development and progression of CRPC (Zhao *et al*. 2016). Although targeting FOXA1 is complicated by its putative dual role in PCa, strategies to suppress GATA2 may have higher feasibility as evidence to date indicates that it plays a positive role in driving CRPC growth.

Alterations to AR corepressors also play a key role in CRPC. For example, loss of activity of the key nuclear receptor corepressors NCOR1 and NCOR2, by either mutation and/or deletion, is relatively common in primary PCa and enriched in CRPC (Grasso *et al*. 2012, Cancer Genome Atlas Research 2015, Robinson *et al*. 2015, Kumar *et al*. 2016). NCORs compete with key AR coactivators, such as p300 and SCR-1, for binding to the ligand-activated receptor, thereby inhibiting its transcriptional activity (Yoon & Wong 2006). Thus, loss of these factors facilitates AR signaling in malignant tissues. Another negative regulator of AR activity, SPOP, was the most commonly mutated factor in an early study of localized PCa (Barbieri *et al*. 2012), and more recent genomics programs of both primary disease and CRPC have validated this finding (Grasso *et al*. 2012, Cancer Genome Atlas Research 2015, Robinson *et al*. 2015, Kumar *et al*. 2016). SPOP is an E3 ligase that promotes the ubiquitination and degradation of AR and SRC-3, reducing the latter's capacity to enhance AR transcriptional activity (Geng *et al*. 2013, 2014). All SPOP mutants identified to date lack the capacity to interact with SRC-3 (Geng *et al*. 2013) or AR itself (An *et al*. 2014, Geng *et al*. 2014), resulting in the stabilization of these substrates. Importantly, the SPOP-binding motif resides in AR's hinge domain; therefore, AR-Vs lacking this region (i.e. AR-V2, AR-V5, AR-V7 and AR-V4, but not ARv567es) can escape SPOP-mediated destruction (An *et al*. 2014).

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New drugs and emerging strategies to target persistent AR signaling driving CRPC

Persistent AR signaling as a driver of CRPC has inspired the hunt for new AR-directed drugs; a subset of agents in clinical development are illustrated in Fig. 2, whereas Supplementary Table 4 comprises a more comprehensive list.

Novel androgen receptor antagonists

Given the recent success of enzalutamide, the search for and development of novel AR-LBD antagonists remains a key priority. One such antagonist is ARN-509, a new-generation anti-androgen with similar structure and mechanism of action to enzalutamide but with potentially increased potency, better pharmacological characteristics and improved patient tolerability (Clegg *et al*. 2012). Although the development of ARN-509 is ongoing, it must be noted that an AR mutation, F877L, that can confer resistance to this agent has already been reported (Joseph *et al*. 2013, Korpal *et al*. 2013). Another promising AR antagonist in clinical development is ODM-201, which is reported to be more potent that enzalutamide in inhibiting AR nuclear translocation (Moilanen *et al*. 2015). Moreover, ODM-201 potentially has the added benefit of activity against AR mutants commonly found in CRPC, namely, T878A, W742L and F877L mutant (Fizazi *et al*. 2014, Moilanen *et al*. 2015). Phase III clinical trials to evaluate the safety and efficacy of ODM-1 in non-metastatic CRPC patients (NCT02200614) and the efficacy of ODM-1 in combination with ADT and docetaxel in patients with metastatic hormone-naïve PCa (NCT02799602) are ongoing.

Targeting androgen receptor expression

Although AR antagonists remain a key focus of research and industry, the realization that ligand-independent forms of the AR, such as mutants and variants, arise in CRPC has elicited novel strategies aimed at suppressing all forms of the AR. In this respect, agents that degrade or inhibit the expression of AR represent a rational approach. One interesting candidate is galeterone, which in addition to promoting the degradation of AR and AR-Vs (Yu *et al*. 2014*a*, Kwegyir-Afful *et al*. 2015) also has activity as an LBD antagonist and an inhibitor of cytochrome P450 17alpha-hydroxylase/17,20-lyase (CYP17), an enzyme essential for the biosynthesis of androgens (Njar & Brodie 2015, Bastos & Antonarakis 2016). Although this proposed 'triple method of action' is exciting, it must be noted that the degrader activity of galeterone is somewhat controversial; it may simply be a by-product of abrogating ligand binding (Yu *et al*. 2014*a*). Notwithstanding these concerns, the putative anti-AR-V activity of galeterone led to the development of a phase III clinical trial (ARMOR3-SV; NCT02438007) in which men with AR-V7-positive disease, as assessed

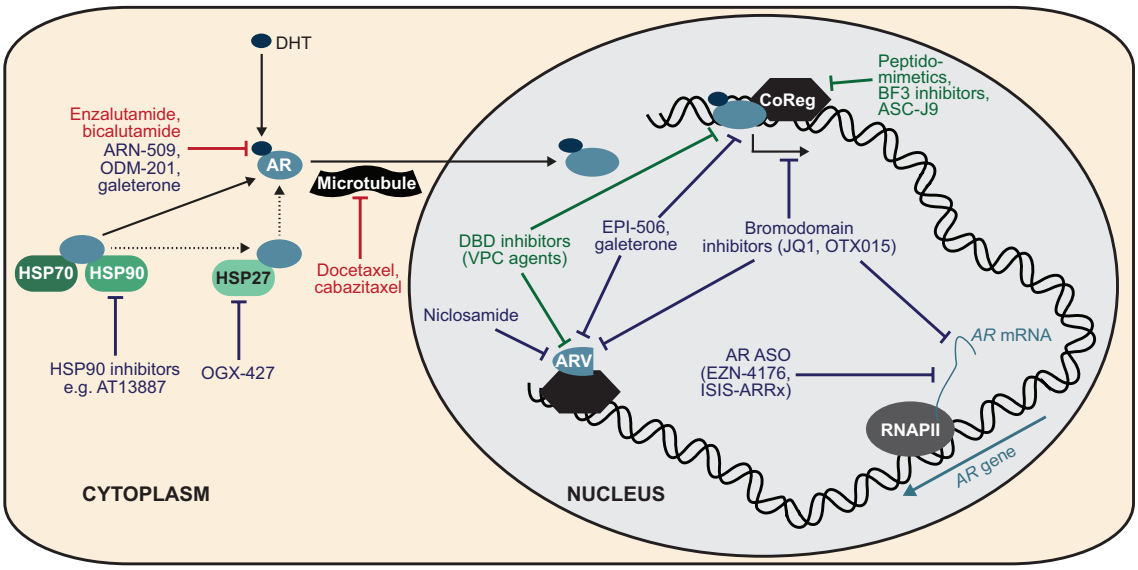

Figure 2
Novel strategies to target persistent androgen receptor signaling in CRPC. Recently approved agents are shown in red; agents in clinical trials are shown in blue; novel agents still in pre-clinical development are shown in green. CoReg, coregulator; HSP, heat shock protein.

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by the AdnaGen assay (Antonarakis *et al*. 2014), were randomized to galeterone or enzalutamide treatment arms. Unfortunately, this trial was discontinued in July 2016 due to lack of an improvement in radiographic progression-free survival for galeterone vs enzalutamide; the fate of this drug is now unclear.

Ligand-bound AR undergoes cycles of ubiquitination and deubiquitination when engaging in its chromatin-associated transcriptional activities, and turnover of polyubiquitinated AR by the ubiquitin–proteasome system (UPS) is important for the stimulation and regulation of AR-driven transcription (Voutsadakis & Papandreou 2012). In addition to SPOP, several other E3 ligases including Siah2 (Qi *et al*. 2013), RNF6 (Xu *et al*. 2009*b*), MDM2 (Lin *et al*. 2002) and CHIP (Sarkar *et al*. 2014) have been shown to mediate AR ubiquitylation, and these factors collectively enable another level of complexity in terms of AR regulation. Elucidating mechanisms underlying AR degradation has facilitated the rational design of proteolysis-targeting chimeras (PROTACs), which link an AR-binding element to a degron tag, such as an E3 ligase-recruiting moiety (Tang *et al*. 2009) or a hydrophobic tag (Gustafson *et al*. 2015), resulting in targeting of the AR to the UPS for degradation. Several AR-targeting PROTACs are being intensively pre-clinically characterized but are yet to reach clinical trials.

Niclosamide was identified in a screen for drugs that inhibit AR-V7 transcriptional activity and specifically causes AR-V7, but not AR-FL, degradation via a proteasome-dependent mechanism (Liu *et al*. 2014*a*, 2015). Importantly, this drug potentiates the effects of enzalutamide *in vitro* and *in vivo* and resensitizes enzalutamide-resistant prostate cancer cells (Liu *et al*. 2014*a*, 2015), findings that have elicited a phase I trial to assess the utility of niclosamide in combination with enzalutamide for treating AR-V7-positive CRPC (NCT02532114).

Inhibition of *AR* gene transcription, as opposed to degradation of AR protein, is another promising therapeutic strategy for the suppression of all forms of the AR that are active CRPC. The recent finding that retinoic acid-related orphan receptor γ (ROR-γ) is a key regulator of *AR* gene transcription led to the pre-clinical assessment of ROR-γ antagonists (Wang *et al*. 2016). These agents effectively reduced the expression and activity of AR-FL and AR-V7, thereby inhibiting tumor growth and metastasis, highlighting the potential of targeting upstream regulators of *AR* gene transcription (Wang *et al*. 2016). Another means to more specifically target *AR* gene expression is the use of antisense oligonucleotides (ASOs). EZN-4176, an ASO that targets exon 4, demonstrated an impressive activity in pre-clinical studies (Zhang *et al*. 2011). Unfortunately, EZN-4176 failed phase I trials in CRPC due to poor *AR* knockdown in the clinical setting and minimal antitumor activity (Bianchini *et al*. 2013). More recently, an exon 1-targeting ASO with improved tissue half-life and activity against enzalutamide-resistant models and CRPC patient-derived xenografts was developed (Yamamoto *et al*. 2015). One advantage of this latter ASO is that targeting exon 1 will theoretically yield activity against all C-terminally truncated forms of the AR and LBD mutants.

Targeting DNA binding and N-terminal functions of AR

Structural knowledge of the AR-LBD, the known 'druggability' of ligand-binding pockets and the relative ease of screening for LBD-inhibiting agents are all reasons for the past focus AR antagonists. By contrast, the development of strategies to block other key functional domains of the AR such as the DBD and NTD is more complicated. Nevertheless, recent progress has yielded promising candidates. For example, two agents designed to block the binding of AR to chromatin, VPC-14228 and VPC-14449, are in early clinical development, with both exhibiting potent anti-transcriptional activity against AR-FL and ARv567es as well as anti-growth activity in enzalutamide-resistant models (Dalal *et al*. 2014, Li *et al*. 2014). Despite the intrinsically disordered nature of the AR-NTD, which has greatly hindered its structural characterization, agents targeting this domain have also been developed. EPI-001 interacts with and covalently binds to the TAU5 domain in the AF-1 region of the AR-NTD (De Mol *et al*. 2016), thereby inhibiting AR:coactivator interactions (e.g. CBP and RAP74) and attenuating AR and AR-V transcriptional activity (Andersen *et al*. 2010, Myung *et al*. 2013). Importantly, EPI-001 enhanced the therapeutic response to docetaxel in CRPC cells harboring both AR-FL and AR-V7, a finding that has implications for combinatorial therapy (Martin *et al*. 2015). Although EPI-001 was reported to selectively inhibit AR over other nuclear receptor family members (Andersen *et al*. 2010), a recent study found that it inhibits the growth of AR-negative cells, at least partly due to off-target effects against proliferator-activated receptor-γ activity (Brand *et al*. 2015). EPI-001 is a mixture of EPI-002, EPI-003, EPI-004 and EPI-005 stereoisomers, and more recent work has focused on elucidating the activities of each with a view

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toward improving target specificity. For example, EPI-002 is reported to have improved anti-tumoral activity compared to EPI-001, with activity in the context of AR coactivator (SRC1-3 or p300) overexpression, gain-of-function AR mutations (both AR-NTD and AR-LBD mutants) and AR-V expression (Yang *et al*. 2016). The oral prodrug form of EPI-002, referred to as EPI-506, displayed strong binding to AR and low toxicity in mice (Myung *et al*. 2013). EPI-506 is currently under phase I/II clinical testing in post-abiraterone and post-enzalutamide settings, with initial results expected in 2017 (NCT02606123).

Targeting androgen receptor coregulators

Given the known dependence of persistent AR signaling on key coregulators, the concept of directly targeting these factors has long been mooted. Recent reviews have described in detail the current state-of-play in terms of targeting key members of the AR interactome (Biron & Bedard 2015, Foley & Mitsiades 2016), including chaperones (HSP90, HSP27 and others), pioneer factors (e.g. FOXA1 and GATA2) and transcriptional coactivators. In this section, we will highlight some key opportunities in this field.

One coregulator-targeted strategy involves directly blocking AR:coregulator interactions. This approach has been achieved using peptides that mimic interaction surfaces. For example, two SRC-1-derived peptides (corresponding to amino acids 1050–1150 and 1050–1240 of SRC-1) effectively inhibited AR-FL and AR-V7 activity by disrupting AR:SRC-1/SRC-2 as well as AR-NTD:AR-LBD interactions (Nakka *et al*. 2013). Although peptides have high specificity and are a cheap and effective screening tool *in vitro*, poor stability *in vivo* limits their clinical utility. Peptidomimetics combine the specificity of peptides with the desirable pharmacological attributes of small molecules, such as stability and bioavailability. The peptidomimetic D2 was designed to mimic the LXXLL motif, which is found in many nuclear receptor coregulators (Ravindranathan *et al*. 2013). D2 effectively disrupted the interaction between AR and the coactivator PELP1, which contains 10 LXXLL motifs, thereby blocking AR nuclear localization and transactivation and prostate cancer growth (Ravindranathan *et al*. 2013). Although numerous other LXXLL-derived peptidomimetic inhibitors of AR:coactivator interactions are in pre-clinical development (Biron & Bedard 2015), it must be noted that targeting this motif may be an ineffective strategy in the case of AR-V-driven disease (Ravindranathan *et al*. 2013). Thus, the elucidation of other protein:protein interaction surfaces and motifs is required to develop novel, selective inhibitors of AR:coregulator interactions.

Strategies to target coregulators that require no prior knowledge of the AR:coregulator interface are also being developed. An exciting example of such an approach is provided by the recent demonstration of potent pre-clinical efficacy of bromodomain and extra-terminal (BET) inhibitors. BET family members bind to acetyl residues on histones and other chromatin-associated factors and thereby act as key transcriptional regulators. Recently, an interaction between the AR-NTD and the BET factor BRD4 was identified (Asangani *et al*. 2014). Disruption of this interaction with a small-molecule inhibitor of BRD4, JQ1, blocked AR chromatin association, transcriptional activity and AR-mediated PCa growth (Asangani *et al*. 2014). More recently, a pan-BET degrader (based on PROTAC technology) was shown to have potent anti-CRPC activity (Raina *et al*. 2016). Importantly, we and others have shown that BET inhibition has a dual mechanism of action, suppressing not only AR transcriptional activity but also *AR* gene transcription (Chan *et al*. 2015, Raina *et al*. 2016). This finding, combined with the mapping of the BRD4 interaction surface to the AR-NTD, suggests that BET inhibitors would have activity in CRPC driven by AR-LBD mutants and/or AR-Vs, a prediction that has been experimentally substantiated (Chan *et al*. 2015, Asangani *et al*. 2016, Raina *et al*. 2016).

Targeting pioneer factors to suppress hormone receptor activity is an emerging concept (Nakshatri & Badve 2007). It must be noted that there is concern about the potential utility of inhibiting FOXA1, given that it can seemingly act to both promote and suppress AR-mediated CRPC growth depending on context (see above). By contrast, a number of factors have converged to make the pioneer factor GATA2 an attractive target (He *et al*. 2014): (i) GATA2 enhances both the expression and activity of AR; (ii) GATA2 is also required for signaling by AR-Vs; (iii) a small-molecule inhibitor of GATA2 (K7174) is available and (iv) GATA2 is reported to have an AR-independent role in driving chemoresistance in prostate cancer (Vidal *et al*. 2015), meaning that its targeting could potentially suppress multiple oncogenic pathways. However, GATA2 plays a key role in multiple aspects of normal physiology, most notably hematopoiesis and angiogenesis, and GATA2 deficiency can result in susceptibility to infections, leukemia and other blood disorders (Hsu *et al*. 2015); any future efforts to develop GATA2 inhibitors for CRPC will need to take this into account.

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Why are prostate cancer cells addicted to androgen receptor signaling?

In the face of a concerted attack in the way of sequential first- and second-line AR-targeted therapies, prostate cancer cells adapt in such a way that AR (in most cases) remains the dominant driver of disease. This exquisite dependence on an individual driver of malignancy is an example of the phenomenon known as oncogene addiction (Weinstein 2002, Pagliarini *et al.* 2015). Although many mechanisms explaining the means by which AR signaling is maintained and/or adapts to circumvent all current forms of AR-targeted therapies in PCa have been elucidated, some of which were described previously, a key question that is rarely explored is simply: what underlies the addiction of PCa to AR?

Answering this question is not straightforward, but an understanding of the role of AR in normal physiology provides clues. Indeed, although this review has focused on the role of AR in prostate cancer, it is important to remember that the androgen/AR signaling axis regulates the development and maintenance of primary and secondary male sexual characteristics, plays a key role in female fertility and also has a plethora of functions in various non-reproductive tissues of both genders (De Gendt & Verhoeven 2012). Given that loss of AR would have dire repercussions for organismal viability, it is logical to expect that cells have developed fail-safe mechanisms to ensure the maintenance of functional AR signaling. It is also worth noting that such mechanisms are likely to be more active in men who have a single X chromosome and hence a single copy of the *AR* gene. In response to AR-targeted therapies, prostate cancer cells could readily 'hijack' this intrinsic capacity to sustain AR activity. One pertinent example of this concept is the capacity of AR to auto-regulate its own expression, which constitutes an important homeostatic mechanism in normal cells but is exploited in CRPC to upregulate AR expression (Cai *et al.* 2011). Further investigation of normal prostate physiology will likely provide key insights into the intrinsic mechanisms underlying persistent AR signaling.

The concept of a 'one-step' remedy for cancers addicted to a specific oncogene (Weinstein 2002) has clearly not been attainable in the case of AR. However, the path to curative therapy can be guided by lessons learnt from both CRPC and other cancers that exhibit unequivocal dependence on a particular oncogene. These lessons have taught us that outcomes can be improved by simply making better inhibitors (e.g. enzalutamide) but that more sophisticated strategies such as 'vertical' and/or 'parallel' combination therapies (Pagliarini *et al.* 2015) will likely be required to achieve substantial gains. Another observation of relevance to this discussion was obtained by Zhang and coworkers who noted that combining two distinct modes of pharmacological inhibition of a single driver oncogene, Bcr-Abl, in chronic myeloid leukemia reduced the occurrence of resistance mechanisms associated with either mode alone (Zhang *et al.* 2010). We favor the idea that combining current ligand-centric strategies with agents that block other aspects of AR function, such as DNA binding or N-terminal transcriptional activity, will be an effective strategy in the future to curtail therapy resistance and thereby reduce the rates of CRPC.

Although this review has focused on persistence of AR signaling in CRPC, we note that trans-differentiation of tumors to an AR-independent, neuroendocrine-like state is an emerging clinical issue, albeit relatively uncommon (Tilki & Evans 2014, Beltran *et al.* 2016). However, as the potency and selectivity of AR inhibitors increase, this disease state may occur more frequently (Beltran 2016). Interestingly, this phenomenon may be generalizable as similar phenotypic transformations have been observed in other tumor types in response to targeted therapies (Sequist *et al.* 2011). Within this context, therapeutic strategies aimed at modulating the activity of AR, as opposed to a dogmatic focus on inhibition, may have benefit: one example is bipolar androgen therapy (Schweizer *et al.* 2015), which could potentially block tumor cell trans-differentiation by sequential stimulation of both AR activation (supraphysiologic testosterone) and inhibition (ADT).

Concluding remarks and future directions

Notwithstanding the recent identification and characterization of AR-independent forms of CRPC (Tilki & Evans 2014, Beltran *et al.* 2016), the majority of CRPC tumors remain driven by persistent AR signaling. Mechanisms underlying this persistence, and potential strategies to target it, were outlined previously. To finish, we list some key outstanding questions and comments that we believe represent key avenues of future research:

- AR signaling-driven castration resistance can be acquired via multiple and convergent events in different metastases, a phenomenon explained by the selective pressure of ADT (Gundem *et al.* 2015). What are the therapeutic consequences of the co-existence of multiple forms of persistent, aberrant AR signaling in

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the same patient? How do we best identify and target the drug-resistant, lethal clones?

- Many different structurally aberrant androgen receptors, such as specific point mutants and truncated variants, have been identified. How do we (comprehensively and rapidly) elucidate the molecular functions of each? Moreover, given the potential for heterodimerization of distinct forms of the AR, how do we optimally model the consequences of interplay between them?
- AR coregulators are essential for the activity of AR in normal cells (Mestayer *et al*. 2003, Xu *et al*. 2009*a*). Can we push AR towards its 'normal' function by shaping, both qualitatively and quantitatively, the AR interactome?
- There is a growing realization that no single AR-targeting drug *per se* will eliminate CRPC due to the constant adaptive evolution of the disease in the face of therapy. Combinatorial therapies have high potential to overcome this issue. However, we urgently require better predictive tools/biomarkers to guide the application of such therapies and identify the patients most likely to receive benefit.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/ERC-16-0422.

Declaration of interest

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

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