Emerging data on androgen receptor splice variants in prostate cancer

Subing Cao¹,², Yang Zhan¹,² and Yan Dong¹,²

¹College of Life Sciences, Jilin University, Changchun, Jilin, China
²Department of Structural and Cellular Biology, Tulane University School of Medicine, Tulane Cancer Center, New Orleans, Louisiana, USA

Abstract

Androgen receptor splice variants are alternatively spliced variants of androgen receptor, which are C-terminally truncated and lack the canonical ligand-binding domain. Accumulating evidence has indicated a significant role of androgen receptor splice variants in mediating resistance of castration-resistant prostate cancer to current therapies and in predicting therapeutic responses. As such, there is an urgent need to target androgen receptor splicing variants for more effective treatment of castration-resistant prostate cancer. Identification of precise and critical targeting points to deactivate androgen receptor splicing variants relies on a deep understanding of how they are generated and the mechanisms of their action. In this review, we will focus on the emerging data on their generation, clinical significance and mechanisms of action as well as the therapeutic influence of these findings.

Introduction


As a steroid receptor, AR activity is not only regulated by ligand binding but also affected by protein–protein interactions, including homodimerization and interactions with cofactors (reviewed in Gelmann 2002, Chan & Dehm 2014). The recruitment and formation of multiple protein complexes are required to activate or repress downstream gene expression. The same rules can be applied to AR-Vs. Several studies indicated that AR-Vs may activate different gene profiles in a cell-context-specific manner (reviewed in Lu et al. 2015). However, the factors that determine the specificity of AR-V-regulated gene sets are currently unknown. One appealing mechanism is that selective gene targeting is achieved by different protein complexes formed between AR-Vs and their partners and/or cofactors, which may induce the formation of novel interfaces to allow the installment of the general transcriptional machinery.
In this review, we summarize the recent discoveries about the mechanisms of AR-V generation, their clinical values, and the modulation of their transcriptional activity by their dimerization partners and cofactors in CRPC.

**AR-V structure**

To date, over 20 AR variants have been identified in human prostate cancer cell models and clinical specimens (Fig. 1). Some of these variants are constitutively active, such as AR-V7 and AR-Vs67es (aka AR-V12), whereas some others are conditionally active, depending on the cellular context, such as AR-V1 and AR-V9 (Hu et al. 2011). Except AR45, which is truncated in the N-terminal domain, all the other variants contain an intact N-terminal domain but lack portions of the ligand-binding domain (LBD). As the N-terminal domain harbors the two trans-activating regions.

**Figure 1**
Schematic representation of the structure of AR-FL and AR-V transcripts and proteins. (A) AR gene structure with canonical exons and the cryptic exons (CE). (B) AR-FL mRNA structure showing exons encoding the N-terminal domain (NTD; exon 1), DNA-binding domain (DBD; exons 2 and 3), hinge region (part of exons 3 and 4) and ligand-binding domain (LBD; exons 5–8). AF-1, Tau1, Tau5/AF-5 and AF-2 are activation function domains. Filled triangle depicts the D-box, which mediates AR-V/AR-V, AR-V/AR-FL and AR-FL/AR-FL dimerization. (C) mRNA and protein structures of AR-Vs. AR-V-specific peptide sequences are indicated in red, and the ‘-’ in AR-V56es indicates a unique junction. Inverted open triangle depicts translation stop. Drawings are not to scale. Exon 9 harbors four cryptic 3′ splicing sites, and the corresponding cryptic exons are indicated as 9a, 9b, 9c and 9d.
(Tau1 and Tau5/AF5), AR45 loses its trans-activating ability and acts as a dominant-negative variant to inhibit the function of the full-length AR (AR-FL) by forming a heterodimer with AR-FL (Ahrens-Fath et al. 2005).

Most AR-Vs contain an intact DNA-binding domain (DBD); however, AR8 does not have a functional DBD, and AR-V3 (aka AR6) lacks the second zinc finger of the DBD. As a result of missing DBD, AR8 cannot function as a transcription factor (Yang et al. 2011). Instead, it primarily locates on the plasma membrane and promotes EGF-induced Src activation and AR-FL phosphorylation and transactivation (Yang et al. 2011). In contrast, AR-V3, which still contains the AR DNA-binding interface residing in the first zinc finger of DBD, can constitutively activate AR-responsive promoters in prostate cancer cells (Dehm et al. 2008). Another variant, AR23, has in-frame insertions between the two zinc fingers by retaining part of the intronic sequence. AR23 was shown to exhibit exclusively cytoplasmic activities, promoting the transcriptional activity of nuclear factor-κB while decreasing the activity of activator protein-1 (Jagla et al. 2007). It is likely that the insertion of the intronic sequence has scrambled the DBD.

Following the DBD is the hinge region, encoded by part of exon 3 and exon 4, which has been shown to harbor the canonical nuclear localization signal. The nuclear localization property is important for the variants to perform their trans-activating function. Although some of the variants do not have the nuclear localization signal, they are still primarily located in the nucleus or have a basal level sufficient for ligand-independent transcriptional activity (Chan et al. 2012). Some mechanisms were proposed for their nuclear localization, such as the existence of a nuclear localization signal-like sequence, lack of nuclear export signal (Saporita et al. 2003, Chan et al. 2012) or tyrosine phosphorylation in the N-terminal domain (Karaca et al. 2015). However, these mechanisms do not appear to satisfactorily cover all the variants with predominant nuclear localization. Characterization of the involved amino acid sequences and understanding the mechanisms that govern their nuclear localization may offer the potential to block AR-V nuclear localization.

**AR-V production**

AR-Vs may arise from multiple mechanisms. Genomic rearrangement of the AR gene has been associated with AR-V generation (Li et al. 2011, 2012b, Nyquist et al. 2013). Modeling gene rearrangement in prostate cancer cells showed expression of AR-V567es without AR-FL in clonally selected cells (Nyquist et al. 2013). Although AR gene rearrangement could contribute to AR-V production in the subset of prostate cancers with AR-Vs being the predominant form of AR expressed, other mechanisms, such as the involvement of specific splicing factors, may underlie the co-expression of AR-FL and AR-Vs observed in many prostate cancer specimens (Miyamoto et al. 2015). Nadiminty and coworkers showed that the splicing factor hnRNPA1 is upregulated and correlated with AR-V7 expression level in 22Rv1 cells with acquired enzalutamide resistance (Nadiminty et al. 2015). The recruitment of hnRNPA1 to the AR-V7 and AR-V3 (aka AR-1/2/2b) splicing sites in AR pre-mRNA is increased, but no significant change is observed in the recruitment of hnRNPA1 to the AR-FL splicing sites, suggesting hnRNPA1 can selectively regulate the generation of AR-Vs (Nadiminty et al. 2015). In addition, Liu and coworkers reported that splicing factors U2AF65 and ASF/SF2 can recognize the binding sites near AR exon 3B and facilitate the recruitment of RNA spliceosome to the AR-V7 3′ splicing site in VCaP and LNCaP95 cells after androgen deprivation (Liu et al. 2014b). Unlike the hnRNPA1 in the aforementioned study, the expression of U2AF65 and ASF/SF2 is not changed after androgen deprivation. Instead, increased AR-V7 production is due to elevated AR pre-mRNA substrates for those splicing factors (Liu et al. 2014b). Furthermore, the study by Ferraldeschi and coworkers implicated a role for the molecular chaperone, HSP90, in AR-V7 splicing (Ferraldeschi et al. 2016). HSP90 inhibition leads to the disruption of AR-V7 splicing and reduction of AR-V7 level (Ferraldeschi et al. 2016). Finally, another study highlighted the interplay between the noncoding RNA PCGEM1 and splicing factors in contributing to AR splicing (Zhang et al. 2016). Androgen deprivation was found to induce PCGEM1 redistribution into nuclear speckles, and the interaction between PCGEM1 and U2AF65 promotes AR-V7 splicing (Zhang et al. 2016). Thus, mechanisms governing AR-V production could be cell-context specific and may involve more layers of regulation beyond splicing factors.

**Clinical relevance**

Accumulating clinical studies showed an association among AR-V expression and prostate cancer progression, therapy resistance, and poor clinical outcome. AR-Vs can be detected in benign prostate tissues, hormone-naïve prostate cancers and CRPC samples, with the most frequent and highest expression detected in CRPC samples (Guo et al. 2009, Hu et al. 2009, Sun et al. 2010, Castagnola et al. 2016). Accumulating evidence indicates that AR-Vs can contribute to androgen independence and potentially mediate resistance to anti-androgens. Understanding the mechanisms underlying AR-V production and their functional implications could provide new targets for therapeutic intervention.
Hornberg et al. 2011, Zhang et al. 2011, Antonarakis et al. 2014, Abeshouse et al. 2015, Qu et al. 2015, Miyamoto et al. 2015, Robinson et al. 2015, Welti et al. 2016. Higher expression of AR-V7 in hormone-naive prostate tumors has been shown to correlate with increased risk of biochemical recurrence after radical prostatectomy (Guo et al. 2009, Hu et al. 2009) and more rapid progression to CRPC (Qu et al. 2015). Moreover, high levels of AR-V7 mRNA or nuclear AR-V7 protein or detectable expression of AR\textsuperscript{v567es} mRNA in CRPCs are associated with a shorter survival of the patients (Hornberg et al. 2011, Qu et al. 2015, Welti et al. 2016). Thus, AR-V expression appears to be associated with a more lethal form of the disease. In support of this clinical evidence, preclinical studies showed that expression of AR-V7 or AR\textsuperscript{v567es} in prostate cancer cell lines or in prostate epithelium of transgenic mice can induce epithelial-to-mesenchymal transition markers (Cottard et al. 2013, Liu et al. 2013, Sun et al. 2014) and castration-resistant growth of prostate cancer cells (Guo et al. 2009, Sun et al. 2010, Watson et al. 2010).

AR-Vs have also been shown to confer both primary and acquired resistance to abiraterone and enzalutamide in preclinical models (Mostaghel et al. 2011, Li et al. 2013, Nadiminty et al. 2013, Cao et al. 2014, Yamamoto et al. 2015). Significantly, AR-V7 has been indicated to have prognostic value in CRPC patients treated with abiraterone or enzalutamide. In a prospective phase 2 study, Efstathiou and coworkers assessed the AR-V7 protein levels in bone marrow biopsies from 60 patients with bone metastatic CRPC before and after enzalutamide treatment (Efstathiou et al. 2015). The presence of AR-V7 is associated with primary resistance to enzalutamide (Efstathiou et al. 2015). In another study, Antonarakis and coworkers evaluated the AR-V7 mRNA level in circulating tumor cells from metastatic CRPC patients before the initiation of enzalutamide or abiraterone treatment (Antonarakis et al. 2014). None of the patients with AR-V7-positive circulating tumor cells showed a response of prostate-specific antigen (PSA) to enzalutamide or abiraterone, and AR-V7 positivity is associated with a shorter progression-free survival of the patients (Antonarakis et al. 2014). Together, these studies suggested the potential of using AR-Vs as a predictive marker of response to enzalutamide and abiraterone.

The role of AR-Vs in therapy resistance may not be limited to androgen-directed therapies. Preclinical studies indicated that AR-Vs might also contribute to taxane resistance. Paclitaxel, docetaxel and cabazitaxel are members of the taxane family of chemotherapeutic agents. They bind to and stabilize microtubules to suppress microtubule dynamics (Martin & Kyprianou 2015, Mellado et al. 2016). Recent literature suggested that androgen-induced AR-FL nuclear import, which is facilitated by microtubule, can be inhibited by paclitaxel and docetaxel (Zhu et al. 2010, Darshan et al. 2011, van Soest et al. 2013, Zhang et al. 2015) but may or may not by cabazitaxel (van Soest et al. 2013, de Leeuw et al. 2015, Zhang et al. 2015, Martin et al. 2016). In contrast to AR-FL, AR-V7 is not only resistant to docetaxel and paclitaxel inhibition of nuclear translocation (Thadani-Mulero et al. 2014, Martin et al. 2015, Zhang et al. 2015) but also attenuates the ability of docetaxel and paclitaxel to retain AR-FL in the cytoplasm (Zhang et al. 2015). The role of AR-V7 in contributing to taxane resistance is further supported by the ability of the AR N-terminal domain antagonist EPI-001/002 to enhance the response of AR-V7-expressing CRPC cells to docetaxel treatment in vitro and in vivo (Martin et al. 2015). However, such role of AR-V7 does not appear to be supported by clinical evidence. Two groups examined AR-V7 mRNA levels in circulating tumor cells from CRPC patients before the initiation of taxane chemotherapy. Both showed that the response to taxanes seems to be independent of the AR-V7 status of circulating tumor cells (Antonarakis et al. 2015, Onstenk et al. 2015). In these studies, the methodologies for isolating circulating tumor cells heavily depend on the expression of an epithelial cell marker on circulating tumor cells (Antonarakis et al. 2015, Onstenk et al. 2015). Whether the exclusion of circulating tumor cells that have undergone epithelial-to-mesenchymal transition in these studies contributes to the conflicting clinical and preclinical findings is yet to be determined.

**Homodimerization and heterodimerization of AR-Vs**

Homodimerization is an essential step for AR-FL to activate target gene expression. The consecutive steps leading to AR-FL homodimerization has been well elucidated (van Royen et al. 2012). Upon ligand binding in the cytoplasm, AR-FL forms intramolecular N-terminal and C-terminal (N/C) interactions, which facilitate nuclear translocation of AR-FL (van Royen et al. 2012). In the nucleus, the intramolecular interactions are followed by a D-box-dimerization-dependent transition to intermolecular N/C interaction (van Royen et al. 2012). Both the intra- and inter-molecular N/C interactions and D-box/D-box interactions are required for AR-FL dimerization (van Royen et al. 2012). Mutations in the...
N-terminal domain or the D-box can both cause the loss of AR-FL transcriptional ability (van Royen et al. 2012). The knowledge on AR-FL transactivation paved the path for the understanding of AR-V transactivation and identification of critical trans-activating steps for therapeutic targeting. We recently showed that, like liganded AR-FL, dimerization is also required for AR-Vs to transactivate target genes. AR-V7 and ARv567es can not only homodimerize but also heterodimerize with each other, and the dimerization is mediated by D-box–D-box interactions (Xu et al. 2015). We further showed that the D-box mutants of AR-V7 and ARv567es lose the ability to transactivate target genes and to induce castration-resistant cell growth (Xu et al. 2015). These findings highlight the potential of targeting AR D-box to inhibit the activity of both AR-FL and AR-Vs for CRPC treatment.

Interplays between AR-Vs and AR-FL

The function of AR-Vs to regulate gene expression has mainly been investigated independent of AR-FL. However, as AR-Vs are often co-expressed with AR-FL in clinical specimens (Miyamoto et al. 2015), the interplays between AR-Vs and AR-FL may be an important mechanism of their actions. Coimmunoprecipitation of ARv567es and AR-FL (Sun et al. 2010) as well as co-occupancy of AR-V7 and AR-FL on the PSA promoter (Cao et al. 2014) indicate that there may be direct interactions between AR-FL and AR-Vs. Using two different assays to detect protein dimerization, we recently showed that both AR-V7 and ARv567es can heterodimerize with AR-FL (Xu et al. 2015). The heterodimerization induces androgen-independent AR-FL nuclear localization and transcriptional activity (Cao et al. 2014). Interestingly, we found that the promoter of the canonical AR target PSA is co-occupied by AR-V7 and AR-FL, whereas the promoter of the UBE2C gene is bound by AR-V7 only (Cao et al. 2014), suggesting that the AR-V/AR-FL dimers and the AR-V/AR-V dimers may regulate different sets of target genes. This is supported by transcriptome and metabolome data showing that AR-Vs can regulate some canonical AR targets as well as a distinct set of genes/pathways (Guo et al. 2009, Hu et al. 2012, Li et al. 2013, Lu et al. 2014, Chan et al. 2015, Shafi et al. 2015). Identifying the respective binding sites for AR-V/AR-FL dimers and AR-V/AR-V dimers across the genome and elucidating whether the genes and pathways regulated by the AR-V/AR-FL dimers fully overlap with those regulated by AR-FL homodimer could be vital in understanding how AR-Vs contribute to castration resistance.

AR-V cofactors

It has long been appreciated that AR cofactors play critical roles in modulating AR activity. However, cofactors for AR-Vs have been scarcely addressed. Although the vast majority of AR-Vs have a truncated C-terminal domain, cofactors that interact with the N-terminal domain of AR-FL may still be involved in modulating the activity of AR-Vs. Several groups have profiled the AR-V transcriptome (Guo et al. 2009, Hu et al. 2012, Li et al. 2013, Lu et al. 2014, Chan et al. 2015), however, AR-V gene expression profiles lack consistency in different prostate cancer models (reviewed in Lu et al. 2015). It is possible that AR-Vs can recruit distinct cofactors that may confer target specificity in different cellular context. As AR-Vs can evade androgen-directed therapies, investigation on the cofactors shared by AR-FL and AR-Vs may provide more potent therapeutic targets.

Theoretically, cofactors bound to the N-terminal domain of AR-FL should interact with AR-Vs as well. Gli2, which binds to the Tau5/AF5 region in the N-terminal domain of AR to enhance AR-FL activity (Chen et al. 2010), can also coactivate AR-V7 and ARv567es (Li et al. 2014b). However, there are also cofactors that interact with AR-FL and AR-Vs through different interfaces and with differing affinity. For example, the transcriptional coactivator FHL2 can increase AR-FL transcriptional activity in an agonist- and AF-2-dependent manner (Muller et al. 2000). As most AR-Vs do not contain the AF-2 domain, it is expected that FHL2 would not influence AR-V activity. However, a recent study showed that FHL2 can also act as an AR-V7 coactivator (McGrath et al. 2013). The authors showed that FHL2 accumulates aberrantly in the nucleus in CRPC cells and that nuclear-accumulated FHL2 directly binds to AR-V7 and enhances its transcriptional activity (McGrath et al. 2013). Although the interacting domain on AR-V7 has not been mapped out, it is clear that FHL2 interacts with AR-FL and AR-V7 through binding to different domains. This case also indicates that AR reactivation in CRPC can be contributed by not only altered expression of cofactors but also deregulated subcellular localization of cofactors. Another example is the cofactor MED1. In the presence of androgen, MED1 has been shown to interact with the LBD of AR-FL and enhance androgen-dependent AR-FL activity.
(Wang et al. 2002). In androgen-deprived condition, PI3K/AKT phosphorylated MED1 (p-MED1) can enhance both AR-FL- and AR\textsuperscript{567es} mediated UBE2C transcription through an enhancer-promoter chromatin-looping mechanism (Wang et al. 2009, Chen et al. 2011, Liu et al. 2015b). However, AR\textsuperscript{567es}, with the assistance of the pioneer factor FOXA1, has been shown to be more potent than AR-FL to recruit p-MED1 to the promoter and enhancer regions of UBE2C (Liu et al. 2015b). This may underlie the preferential regulation of UBE2C by AR-Vs over AR-FL in cells co-expressing AR-FL and AR-Vs (Hu et al. 2012, Cao et al. 2014). Together, these findings suggest that MED1 could not only regulate AR-FL activity through different mechanisms when androgen is present vs absent but also contribute to a switch from AR-FL signaling to AR-V signaling.

AR-FL and AR-Vs may compete for binding to certain cofactors in different biological conditions, leading to the activation of different sets of genes. The RhoGTPase guanine nucleotide exchange factor, Vav3, found to be an AR-FL coactivator (Rao et al. 2012), was shown to also enhance AR-V7 and AR\textsuperscript{567es} transactivation and increase AR-V7 nuclear localization by directly binding to AR-V7 (Peacock et al. 2012). Immunoprecipitation assay showed that AR-V7 may compete with AR-FL for interacting with Vav3 (Peacock et al. 2012).

Some cofactors may regulate not only the activity but also the abundance of AR-FL and AR-Vs. The pioneer factor GATA-binding protein 2 (GATA2) is reported to facilitate AR-FL genomic binding by colocalizing with FOXA1 at the enhancer regions of target genes to produce an accessible chromatin environment for AR-FL before androgen stimulation and by recruiting MED1 to sustain basal enhancer-promoter chromatin looping in the absence of androgen (Wang et al. 2007, He et al. 2014, Wu et al. 2014). It has also been shown to interact with AR-V7 and colocalize with AR-Vs on chromatin (He et al. 2014). At the same time, GATA2 induces the transcription of the AR gene and thereby the levels of both AR-FL and AR-Vs through binding to an extended promoter region of the AR gene (Wang et al. 2007, Bohm et al. 2009, He et al. 2014, Wu et al. 2014). Interestingly, androgen-bound AR represses GATA2 expression (He et al. 2014). Under androgen-deprived condition, with the disruption of this negative-feedback regulatory loop, GATA2 contributes to both overexpression and increased activity of AR-FL and AR-Vs (He et al. 2014). As a result, inhibiting GATA2 has dual efficacy to inactivate AR signaling by ablating both the expression and transcriptional activities of AR-FL and AR-Vs (He et al. 2014). Preclinical data showed that the small molecule inhibitor of GATA2, K7174, significantly decreased the viability of various GATA2+/AR+ prostate cancer cell lines \textit{in vitro} and inhibited the growth of LNCaP- abl xenograft tumors (He et al. 2014). All these data suggested a promising role for GATA2 inhibitors in CRPC treatment.

When coexisting in the same cells, corepressors and coactivators may compete for AR-V binding. FOXO1 is a well-known corepressor of AR (Dong et al. 2006, Fan et al. 2007, Liu et al. 2008, Attard et al. 2009). It can inhibit androgen-independent activation of AR-FL (Liu et al. 2008) and the constitutive activity of different AR-Vs (Bohrer et al. 2013, Mediwala et al. 2013). In addition, FOXO1 represses SRC-1-enhanced transcriptional activity of AR-V5 by competing with SRC-1 for binding to the Tau5/AF5 region of AR-V5. Although the Tau5/AF5 region is present in almost all AR-Vs, SRC-1 can only selectively enhance the transcriptional activity of AR-V5 but not the other variants (Bohrer et al. 2013), indicating that the transactivation of different AR-Vs may involve distinct cofactors and mechanisms. It is also possible that different AR-V dimers may recruit specific cofactors to activate gene expression. Further mechanistic study of how these cofactors contribute to CRPC and their interplay with AR-Vs would drive the development of strategies that effectively target the cofactors.

**AR-V7 degradation**

In addition to blocking the production and activity of AR-Vs, inducing AR-V protein degradation is another attractive approach to suppress AR-V signaling. Li and coworkers recently showed that, similar to AR-FL, the AR-V7 protein can be degraded through an Mdm2-mediated ubiquitin-proteasome degradation process and that this process can be accelerated by Akt signaling but is repressed by protein phosphatase-1 (PP-1) (Li et al. 2015b). Co-targeting the AR and Akt signaling pathways is being tested for CRPC treatment. Would co-suppressing the two pathways lead to a more robust induction of AR-Vs and AR-V-driven tumor progression? If so, would co-targeting the Akt pathway with an agent that can induce AR-V degradation (Li et al. 2012a, Yamashita et al. 2012, Zengerling et al. 2012, Cao et al. 2013, Liu et al. 2014a, Yu et al. 2014, Kwegyir-Afful et al. 2015, Sun et al. 2015) alleviate this problem? These questions need to be carefully addressed.
Therapeutic targeting of AR-Vs

Various approaches have been explored to disrupt AR-V signaling, such as targeting AR N-terminal domain (Myung et al. 2013) or DNA-binding interface (Dalal et al. 2014, Li et al. 2014a), inducing AR-V protein degradation (Li et al. 2012a, Yamashita et al. 2012, Zengerling et al. 2012, Cao et al. 2013, Liu et al. 2014a, Yu et al. 2014, Kwegyr-Afful et al. 2015, Sun et al. 2015), reducing AR-V expression (Mashima et al. 2010, Zhan et al. 2013), inhibiting AR-V chromatin binding (Chan et al. 2015, Li et al. 2015a), disrupting AR-FL and AR-V dimerization (Streicher et al. 2014) or using antisense oligonucleotides against exons shared by AR-FL and AR-Vs (Yamamoto et al. 2015). Here, we describe several AR-V-targeting agents that have entered clinical trials for cancer treatment. EPI-001/002 is a small-molecule, non-steroidal AR antagonist that covalently binds to AR N-terminal domain to inhibit the transcriptional activities of AR-FL and AR-Vs (Andersen et al. 2010, Sadar 2011, Myung et al. 2013, Kato et al. 2016). It has also been found to act as a selective PPARγ modulator to inhibit AR expression (Brand et al. 2015). EPI-001/002 showed excellent anti-tumor efficacy in various preclinical models of CRPC, and its successor, EPI-056, is being investigated in a phase I/II study in metastatic CRPC patients (Andersen et al. 2010, Sadar 2011, Myung et al. 2013, Brand et al. 2015, Martin and Kyprianou 2015).

Galeterone is another small molecule that can inhibit AR-V activity. It was shown to target AR signaling at three levels, blocking androgen synthesis by inhibiting CYP17, inducing proteasomal degradation of AR-FL and AR-V7 and preventing the binding of androgen to the AR (Handratta et al. 2005, Purushottamachar et al. 2013, Yu et al. 2014, Kwegyr-Afful et al. 2015, Njar & Brodie 2015). A Phase III trial comparing galeterone to enzalutamide in treatment-naïve metastatic CRPC patients with AR-V7-positive prostate tumors was launched in 2015. Unfortunately, the trial was terminated in July 2016 due to the unlikelihood of galeterone to show improved radiographic progression-free survival in these patients than enzalutamide. Further clinical testing is being planned in metastatic CRPC patients who rapidly progress on enzalutamide or abiraterone or have developed acquired resistance to enzalutamide.

Niclosamide is an FDA-approved anti-helminthic drug that was found to inhibit AR-V7 expression and activity (Liu et al. 2014a). It also targets the IL6-Stat3-AR axis (Liu et al. 2015a). Niclosamide inhibits prostate cancer cell growth and overcomes enzalutamide and abiraterone resistance in preclinical models (Liu et al. 2014a, 2015a, 2016). These promising preclinical data led to the Phase I trial of niclosamide in combination with enzalutamide in AR-V-positive metastatic CRPC patients.

Several second-generation HSP90 inhibitors are in active clinical trials for cancer treatment. HSP90 interacts with the LBD of AR-FL as a molecular chaperone, which is critical for proper folding, hormone binding and transcriptional activity of AR-FL (Vanaja et al. 2002, Ai et al. 2009). It does not affect the activities of AR-Vs (Vanaja et al. 2002, Shafi et al. 2013). However, it has been reported to play a role in AR-V7 splicing (Ferraldeschi et al. 2016). The second-generation HSP90 Inhibitor onalespib was shown to block AR-V7 mRNA splicing and reduce AR-V7 level, supporting further clinical investigation of HSP90 inhibitors against AR-V7-expressing CRPC (Ferraldeschi et al. 2016).

Several bromodomain and extra-terminal domain family (BET) inhibitors have shown promising efficacies in preclinical studies and are currently under clinical trials in CRPC patients (Asangani et al. 2014). Many AR cofactors contain a bromodomain(s), which recognizes acetylated lysine residues and is essential for chromatin binding and remodeling as well as coactivation. A BET inhibitor, JQ1, was shown to disrupt the recruitment of both AR-FL and AR-Vs to target gene loci and block their transactivation (Asangani et al. 2014, Chan et al. 2015). Although it is likely that not all of these agents can be translated into the clinic, these emerging drugs provided promising and innovative approaches to effectively target AR-Vs for treating CRPC.

Conclusion

The seminar discovery of AR-Vs has revolutionized the field of prostate cancer. It not only broadened our view of how to tackle the AR signaling axis but also provided a most promising prognostic marker for predicting responses of CRPC to current androgen-directed therapies. Various approaches have been explored to disrupt both AR-FL and AR-V signaling. Several emerging drugs, such as EPI-001/002, galeterone, niclosamide, HSP90 inhibitors and BET inhibitors, have shown excellent efficacies to overcome resistance of CRPC to current therapies in preclinical models and are currently under clinical investigation. These innovative approaches of AR targeting have marked the beginning of a new era in prostate cancer treatment. Further investigations of the mechanisms of how AR-Vs are activated to regulate downstream genes and crosstalk with other signaling pathways will continue.
to benefit the search for more effective therapeutic approaches and targets. Finally, the prognostic influence of AR-Vs may allow the implementation of a tailored therapeutic strategy in selecting patients who may benefit from specific treatment at specific point of disease progression. This will be a major step forward in precision medicine for advanced prostate cancer.

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

Funding
This work was supported by the following grants: NIH/NCI R01CA188609; DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485 and National Natural Science Foundation of China Projects 81272851 and 81430087.

References
Ahrens-Fath I, Politz O, Geserick C & Haendler B 2005 Androgen
References
and National Natural Science Foundation of China Projects 81272851
DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485
This work was supported by the following grants: NIH/NCI R01CA188609;
Funding
The authors declare no conflict of interest that could be perceived as
Declaration of interest
The authors declare no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding
This work was supported by the following grants: NIH/NCI R01CA188609; DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485 and National Natural Science Foundation of China Projects 81272851 and 81430087.

References
Ahrens-Fath I, Politz O, Geserick C & Haendler B 2005 Androgen
References
and National Natural Science Foundation of China Projects 81272851
DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485
This work was supported by the following grants: NIH/NCI R01CA188609; DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485 and National Natural Science Foundation of China Projects 81272851 and 81430087.

References
Ahrens-Fath I, Politz O, Geserick C & Haendler B 2005 Androgen
References
and National Natural Science Foundation of China Projects 81272851
DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485
This work was supported by the following grants: NIH/NCI R01CA188609; DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485 and National Natural Science Foundation of China Projects 81272851 and 81430087.

References
Ahrens-Fath I, Politz O, Geserick C & Haendler B 2005 Androgen
References
and National Natural Science Foundation of China Projects 81272851
DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485
This work was supported by the following grants: NIH/NCI R01CA188609; DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485 and National Natural Science Foundation of China Projects 81272851 and 81430087.

References
Ahrens-Fath I, Politz O, Geserick C & Haendler B 2005 Androgen
References
and National Natural Science Foundation of China Projects 81272851
DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485
This work was supported by the following grants: NIH/NCI R01CA188609; DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485 and National Natural Science Foundation of China Projects 81272851 and 81430087.

References
Ahrens-Fath I, Politz O, Geserick C & Haendler B 2005 Androgen
References
and National Natural Science Foundation of China Projects 81272851
DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485
This work was supported by the following grants: NIH/NCI R01CA188609; DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485 and National Natural Science Foundation of China Projects 81272851 and 81430087.

References
Ahrens-Fath I, Politz O, Geserick C & Haendler B 2005 Androgen
References
and National Natural Science Foundation of China Projects 81272851
DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485
This work was supported by the following grants: NIH/NCI R01CA188609; DOD W81XWH-15-1-0439, W81XWH-16-1-0317 and W81XWH-14-1-0485 and National Natural Science Foundation of China Projects 81272851 and 81430087.
coactivator recruitment to the androgen receptor complex. PNAS 111 18261–18266. (doi:10.1073/pnas.1421415111)
AR-Vs in prostate cancer

Mashima T, Okabe S & Seimiya H 2010 Pharmacological targeting of


Njar VC & Brodie AM 2015 Discovery and development of Galertere (TOK-001 or VN/124-1) for the treatment of all stages of prostate cancer. Journal of Medicinal Chemistry 58 2077–2087. (doi:10.1021/acs.jmedchem.5b01239)


Received in final form 27 September 2016
Accepted 4 October 2016
Accepted Preprint published online 4 October 2016