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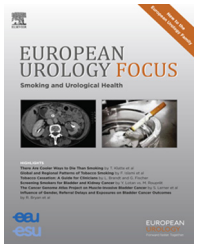


Review – Prostate Cancer

# Understanding Mechanisms of Resistance in Metastatic Castration-resistant Prostate Cancer: The Role of the Androgen Receptor

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## Abstract

***Context:*** After initiation of androgen deprivation therapy (ADT), most patients progress to castration-resistant prostate cancer (CRPC) within 2 or 3 yr. In the USA, approximately 67 000 men are estimated to have metastatic CRPC.
***Objective:*** To provide an overview of different mechanisms driving resistance to therapy in metastatic CRPC, with a focus on androgen receptor (AR)–dependent pathways.
***Evidence acquisition:*** A Medline search via PubMed was performed using the keywords *metastatic castration resistant prostate cancer (mCRPC)*, *castration-resistant*, *CRPC*, *prostate cancer*, *androgen resistance*, *hormone-refractory*, *hormone-independent*, *androgen receptor*, *and androgen receptor axis*. Only articles in the English language were included. Abstracts and full-text articles were reviewed and assessed for relevant content. The majority of the articles selected were published between 1993 and 2016. Older studies were included selectively if relevant.
***Evidence synthesis:*** Numerous resistance mechanisms characterize the development of CRPC. The review focuses on AR-dependent pathways, including mechanisms of resistance to new agents. These include reactivation of AR (via AR amplification, mutations, or splice variants), stress-activated pathways, and aberrant activation of AR.
***Conclusions:*** Mechanisms of resistance in CRPC are manifold and require multiple combinations of therapeutic approaches to be overcome. An understanding of the mechanisms by which resistance to ADT develops is the basis for identifying future therapeutic targets.
***Patient summary:*** Castration-resistant prostate cancer is characterized by multiple resistance mechanisms to androgen deprivation treatment and remains an incurable disease. An understanding of the mechanisms underlying this resistance is necessary to identify future therapeutic targets.


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## 1. Introduction

Prostate cancer remains one of the leading causes of cancer death worldwide [1]. In the era of prostate-specific antigen (PSA) screening, the majority of cancers detected are localized and can be cured. In locally advanced and metastatic prostate cancer, treatment consists of androgen deprivation therapy (ADT), which has been the standard of care since Huggins and Hodges first introduced the concept that prostate cancer is an androgen-dependent disease [2]. However, ultimately all patients will progress to castration-resistant prostate cancer (CRPC), which usually occurs within a few months to 2–3 yr of initiation of ADT. The mechanisms driving the emergence of the CRPC state have been elusive; however, within the last decade it has become clear that androgens and the androgen receptor (AR) are crucial drivers of CRPC. Several resistance mechanisms including reactivation of AR (via AR amplification, mutations, or variants), activation of AR via aberrant pathways, and intratumoral or alternative androgen production have been described. New agents approved for treatment of CRPC such as enzalutamide and abiraterone acetate target a subset of these resistance mechanisms. However, mechanisms of resistance evolve over time against these new agents as well.

The aim of this review is to provide an overview of different mechanisms of treatment resistance in metastatic CRPC (mCRPC) with a focus on AR-dependent pathways.

## 2. Evidence acquisition

A literature review was performed by searching the electronic PubMed/Medline databases. The search was performed using combinations of the following terms: *metastatic castration resistant prostate cancer (mCRPC)*, *castration-resistant*, *CRPC*, *prostate cancer*, *androgen resistance*, *hormone-refractory*, *hormone-independent*, *androgen receptor*, and *androgen receptor axis*. Articles (only English) were selected based on the title, abstract, study format, and content by consensus among the authors. In addition, guidelines from the European Association of Urology (EAU) and American Urological Association were studied to identify relevant studies and recommendations. References from selected studies were reviewed manually. The majority of the articles selected were published between 1993 and 2016. Older studies were included selectively if historically relevant.

## 3. Evidence synthesis

### 3.1. AR

The AR is a ligand-inducible transcription factor of the nuclear receptor superfamily [3]. It consists of a polymorphic N-terminal domain (NTD), a DNA-binding domain (DBD), a small hinge region, and a C-terminal ligand-binding domain (LBD; Fig. 1) [3,4] AR exon 1 encodes the entire NTD, which comprises the bulk of the AR and is the least conserved of the four domains [5]. The *AR* gene is located on the X chromosome at Xq11-12 and is therefore single-copy in males, which allows for phenotypic manifestation of mutations without the influence of a wild-type codominant allele [6]. The unliganded AR associates with a heat shock protein 90 (HSP90) chaperone complex in the cytoplasm and undergoes proteasome-mediated degradation in the absence of ligand [7].

Binding of androgens (testosterone or dihydrotestosterone) to AR results in dissociation of the AR-HSP complex, nuclear translocation, and dimerization. The AR dimer binds to androgen response elements (AREs) in the promoter regions of target genes, and recruits cofactors for regulation of the expression of androgen-regulated genes [6]. The AR is subject to multiple post-translational modifications in response to agonist binding, which include phosphorylation, methylation, acetylation, ubiquitylation, and sumoylation [7].

### 3.2. ADT and castration resistance

ADT is a mainstay in the treatment of metastatic prostate cancer. Testosterone is the main source of circulating androgens in males. The goal of ADT is to reduce serum testosterone to castrate levels, thus inducing regression of the tumor [8]. This approach was based on the important insight by Huggins and Hodges in 1941 that prostate cancer is androgen-dependent [2].

The upper limit of castration concentrations of serum testosterone has been considered to be 50 ng/dl (1.7 nmol/l), although lower concentrations (20 ng/dl; 1 nmol/l) may be more desirable for optimal therapy [9]. In the current EAU guidelines, the castration level is defined as a testosterone concentration of $<$20 ng/dl, and new methods demonstrated a testosterone level of 15ng/dl after surgical castration [1].

ADT can be achieved via either medical or surgical castration [1]. Luteinizing hormone–releasing hormone (LHRH) agonists and antagonists suppress the production of LH via negative feedback or competitive inhibition, and thus suppress testicular testosterone production [10]. Antiandrogens are competitive inhibitors of AR and block the androgen effect. Two different types of antiandrogen exist, nonsteroidal antiandrogens and steroidal antiandrogens, which are derivatives of hydroxyprogesterone.

Sun et al [11] retrospectively evaluated 3295 men who received ADT via orchiectomy or LHRH agonists. The authors noted a lower risk of any fractures in the surgical castration compared to the medical castration group. At 1 yr after prostate cancer diagnosis, there was no significant difference in median total expenditure between surgical castration ($9726.98) and LHRH agonists ($8478.46). They concluded that surgical castration is underutilized and should be considered more frequently in the routine care of patients with metastatic disease [11].

Data from a Southwest Oncology Group (SWOG) trial identified the PSA response after 7 mo of ADT as an independent predictor of survival. The median survival was 13 mo for patients with PSA $>$4 ng/ml, 44 mo for patients with PSA of 0.2–4 ng/ml, and 75 mo for patients with PSA $<$0.2 ng/ml [12].

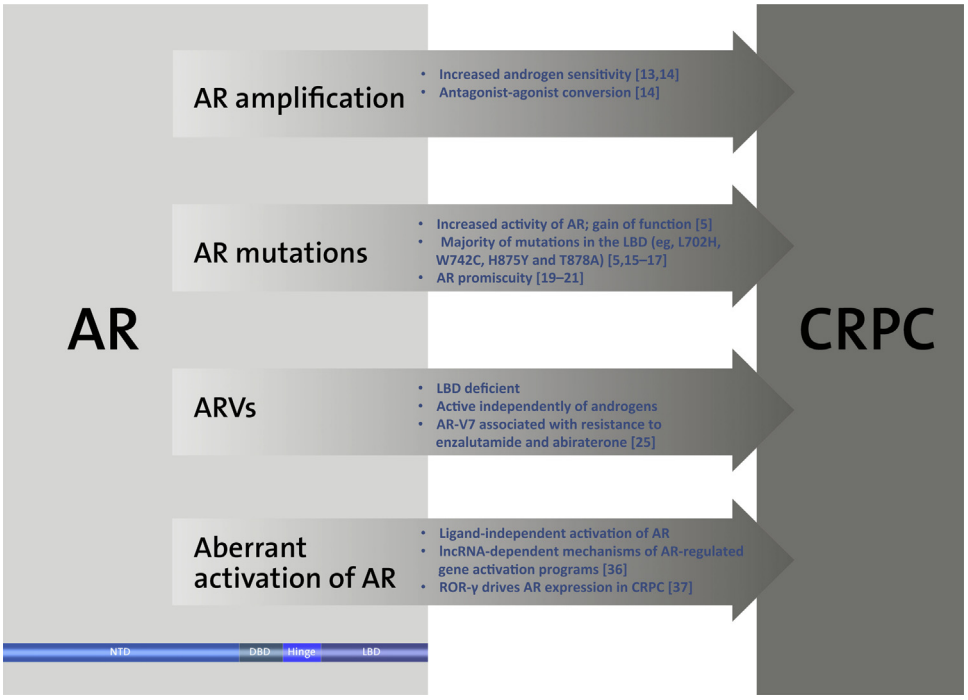


**Fig. 1 – Overview of different androgen receptor (AR)-dependent mechanisms driving resistance in metastatic castration-resistant prostate cancer (CRPC). NTD = N-terminal domain; DBD = DNA-binding domain; LBD = ligand-binding domain; ARVs = AR splice variants.**

Despite an initial response, eventual progression to CRPC is nearly universal. Prostate cancer cells survive and continue to grow despite ADT via adaptation to androgen depletion conditions and alternative survival and growth pathways. Androgens and AR remain crucial drivers of CRPC.

The EAU guidelines define CRPC as castrate serum testosterone <50 ng/dl or 1.7 nmol/l and either biochemical progression (three consecutive rises in PSA 1 wk apart resulting in two 50% increases over the nadir, and PSA >2 ng/ml) or radiologic progression with the appearance of new lesions [1]. Symptomatic progression alone is not sufficient to diagnose CRPC [1].

### 3.3. *Mechanisms of castration resistance*

#### 3.3.1. *AR amplification*

Despite ADT, cells can acquire hypersensitivity to residual low androgen levels (hypersensitivity pathway). In 1995, Visakorpi and colleagues [13] first described AR amplification as a cause of resistance to ADT. The authors studied 23 recurrent tumor specimens from patients treated with ADT and found that 30% had specific high-level amplification of the *AR* gene, while this was not found in untreated tumor samples from the same patients available for 16 of the 23 cases.

Chen et al [14] used microarray-based profiling of isogenic prostate cancer xenograft models (seven hormone-sensitive and hormone-refractory human prostate cancer xenograft pairs) and found that an increase in *AR* mRNA was consistently associated with the development of resistance to ADT. This increase in AR was sufficient to convert prostate cancer growth from a castration-sensitive to a castration-resistant stage. Another finding was that the mechanism by which increased AR levels cause castration-resistant disease was ligand-dependent. High levels of AR sensitize the cell to residual amounts of ligand remaining after ADT [14]. Furthermore, the authors found that AR antagonists showed agonistic activity in cells with increased AR levels (antagonist-agonist conversion) that was associated with alterations in the recruitment of co-activators and co-repressors to the promoters of AR target genes [14].

#### 3.3.2. *AR mutations (AR promiscuity)*

AR mutations that lead to increased AR activity in the presence of low androgen levels rarely occur in the early stages of prostate cancer, while approximately 10–30% of CRPC patients carry gain-of-function AR mutations, especially among patients treated with ADT [5]. AR point mutations were thought to be uncommon, but deep sequencing studies have revealed that these mutations may be more common than once thought [15].

Most of the AR mutations identified in prostate cancer tissue consist of single-base substitutions due to somatic rather than germline mutations. The majority of the mutations identified in prostate cancer patients occur in the LBD [5].

Grasso et al [16] sequenced the exomes for 50 lethal, heavily pretreated metastatic CRPCs obtained at rapid autopsy and 11 treatment naïve, high-grade localized prostate cancers. They found that *AR* is among the genes that are most frequently mutated in mCRPC.

Using a novel next-generation sequencing platform, Beltran et al [15] analyzed archival formalin-fixed,

paraffin-embedded tissue samples (including prostatectomies and prostate needle biopsies) from 45 patients with localized, metastatic hormone-naive PC and mCRPC. The authors reported that 44% of CRPCs harbored genomic alterations involving the AR, including AR copy number gain (24% of CRPCs) or AR point mutation (20% of CRPCs). The AR mutations included three known activating point mutations involving the LBD, as well as a novel variant involving the regulatory domain. Interestingly, the point mutations identified were mutually exclusive of AR amplification (24%) [15].

Recurrent point mutations in the LBD described in several studies include L702H, W742C, H875Y, and T878A. These AR mutations are present in approximately 15–20% of CRPC cases [15,17]. AR mutations can lead to the paradox phenomenon whereby AR antagonists behave as agonists. Hara et al [18] described the mutations W741C and W741L in the LBD after culturing novel LNCaP cell sublines in androgen-depleted medium with bicalutamide to mimic the combined androgen blockade, and showed that bicalutamide worked as an agonist for both W741C and W741L mutant ARs.

Furthermore, AR mutations can lead to AR activation by molecules other than androgens (AR promiscuity). One of the most frequently observed mutants, T877A, binds to other steroid hormones, such as progesterone and estrogens, and to antiandrogens that are converted to agonists [19–21].

Taplin et al [22] initially reported the H874Y mutation, which generated AR that can be stimulated by estrogen and progesterone. Duff and McEwan [23] introduced selected mutations including H874Y into the isolated AR LBD or full-length AR to investigate receptor-structure function relationships. They found that the mutation permits a wider range of steroids and nonsteroid ligands to act as agonists. In addition, they found that this mutation led to enhanced co-activator interactions (members of the p160 co-activator family) and transactivation activity [23]. Different co-regulator complexes that can function as enhancers (co-activators) or repressors (co-repressors) of transcriptional activity are recruited by the AR and serve as modulators of other protein complexes, as well as molecular chaperones and RNA splicing regulators [10].

#### 3.3.3. *AR splice variants*

Another mechanism underlying castration resistance is alternative splicing of *AR* mRNA. AR splice variants (ARVs) are characterized by truncation or exon skipping of the carboxy-terminal LBD. An intact LBD allows androgen-dependent regulation of AR via binding of an androgen, which induces release of AR from HSP90 and nuclear translocation. By contrast, loss of the LBD allows ARVs to be active independently of androgens. To date, numerous ARVs have been described in different cell lines and in clinical samples.

Tepper and colleagues [24] identified a mutant AR in the hormone-insensitive prostate cancer cell line CWR22Rv1 that contains an in-frame tandem duplication of exon 3 that encodes the second zinc finger of the AR DBD and leads to a COOH-terminally truncated AR species migrating with a relative mass of 75–80 kDa that remains active.

AR-V7 (or AR3), one of the best known ARVs, lacks the LBD and can be measured in circulating tumor cells via quantitative reverse transcriptase–polymerase chain reaction assay [25]. Guo et al [26] performed immunohistochemistry analyses on tissue microarrays containing 429 human prostate tissue samples and showed that AR-V7 is significantly upregulated during prostate cancer progression and its expression level is correlated with the risk of tumor recurrence after radical prostatectomy.

Sun et al [27] identified and characterized AR-V567es and demonstrated that this ARV can contribute to cancer progression in human prostate cancer xenograft models following castration. Furthermore, AR-V567es was frequently found in human prostate cancer metastases [27]. Similarly, Hörnberg et al [28] reported detection of AR-V567es transcripts in 23% of CRPC bone metastases. Furthermore they found that its expression was associated with poorer prognosis.

A novel and structurally different ARV that is upregulated in CRPC cells, AR8, was identified by Yang et al [29]. It lacks a DBD and was reported to possibly contribute to castration resistance by potentiating AR-mediated proliferative and survival responses to hormones and growth factors.

Kohli et al [30] analyzed 82 mCRPC patients who underwent metastatic site biopsies before prechemotherapy abiraterone acetate/prednisone and after 12 wk of treatment. Increased *ARV9* mRNA expression in metastases was associated with resistance to abiraterone acetate/prednisone.

#### 3.3.4. *Aberrant AR activation*

Ligand-independent AR activation represents an important mechanism for progression to castration resistance. Ligand-independent mechanisms of AR activation and altered AR transcriptional activity include AR activation by growth factors such as IGF-1, KGF, EGF [31], the receptor tyrosine kinase–activated pathway (HER-2/neu signaling cascade; Src kinase) [32–34], the AKT pathway [35] and lncRNA-dependent mechanisms of AR-regulated gene activation programs [36].

Yang et al [36] reported that two lncRNAs, PRNCR1 (also known as PCAT8) and PCGEM1, are overexpressed in aggressive prostate cancer, bind to AR, and enhance both ligand-dependent and ligand-independent AR-mediated gene activation programs and prostate cancer cell proliferation.

Wang et al [37] found that ROR-γ, a RAR-related orphan receptor, is highly overexpressed in tumors from mCRPC patients and functions as a key determinant of AR overexpression and aberrant signaling in mCRPC tumors. They found that ROR-γ directly stimulates *AR* gene transcription by binding to an exonic RORE and partly through the NR co-activators SRC-1 and SRC-3. Moreover, the authors reported that ROR-γ–selective antagonists inhibit *AR* gene expression, AR genome-wide binding, and

growth of mCRPC cell lines in vitro and in mouse xenografts, and thus provides an opportunity for therapeutic intervention in CRPC [37].

### 3.4. Mechanisms of resistance against new agents available for treatment of CRPC

Mechanisms of castration resistance have been extensively studied during the last decade, which has led to the development of new therapeutic options including abiraterone acetate and enzalutamide, which are approved for use in men with mCRPC [38–42].

Abiraterone is an irreversible inhibitor of CYP17A1, an important enzyme in the synthesis of testosterone and estrogen, and its inhibition effectively suppresses nongonadal androgen and estrogen synthesis [38]. A survival benefit of abiraterone/prednisone over placebo/prednisone has been demonstrated in patients who had progressed on docetaxel therapy and in the prechemotherapy setting [39,40].

Enzalutamide is an AR signaling inhibitor with higher affinity for the AR than bicalutamide. It binds to the LBD of AR and inhibits its nuclear translocation, DNA binding to AREs, and transactivation of recruitment co-activators [43]. Data from the PREVAIL trial demonstrated that enzalutamide significantly decreased the risk of radiographic progression and death, and delayed the initiation of chemotherapy in men with progressive metastatic prostate cancer on ADT [44,45].

Despite these advances, resistance mechanisms evolve over time against these new agents as well.

Antonorakis et al [25] evaluated AR-V7 in circulating tumor cells from prospectively enrolled patients with mCRPC who were initiating treatment with either enzalutamide ($n = 31$) or abiraterone ($n = 31$). They found that detection of AR-V7 in circulating tumor cells was associated with resistance to enzalutamide and abiraterone. However, while Qu et al [46] also found that patients with higher AR-V7 transcript levels had a shorter time to treatment failure on enzalutamide, they could not confirm a significant association between higher AR-V7 and shorter time to treatment failure on abiraterone acetate.

Several other studies have found an association between ARVs, especially AR-V7, and the development of enzalutamide resistance [47–50]. Nadiminty et al [50] reported that resistance to enzalutamide in LNCaP C4–2B and CWR22Rv1 may be mediated by NF-κB2/p52 via activation of AR and its splice variants.

Liu and colleagues [51] performed drug screening using a luciferase activity assay to determine AR-V7 activity after treatment with compounds in the Prestwick Chemical Library, which contains more than 1000 drugs approved by the US Food and Drug Administration (FDA). Niclosamide, an FDA-approved antihelminthic drug, was identified as a potent AR-V7 inhibitor in prostate cancer cells. It significantly downregulated AR-V7 protein expression via protein degradation through a proteasome-dependent pathway. It inhibited prostate cancer cell growth in vitro and tumor growth in vivo [51]. The combination of niclosamide and enzalutamide significantly inhibited enzalutamide-resistant tumor growth, suggesting that niclosamide enhances enzalutamide therapy and overcomes enzalutamide resistance in CRPC cells [51].

Liu et al [52] recently identified AKR1C3, an enzyme in the steroidogenesis pathway, as a mechanism driving resistance to abiraterone acetate via increased intracrine androgen synthesis and enhanced androgen signaling. The authors reported that AKR1C3 overexpression confers resistance to abiraterone, while AKR1C3 downregulation resensitizes resistant cells to abiraterone treatment [52]. Moreover, the study provided evidence that treatment of abiraterone-resistant cells with indomethacin (an AKR1C3 inhibitor) overcame resistance and enhanced abiraterone therapy by reducing intracrine androgen levels and diminishing AR transcriptional activity [52]. Similarly, it has been shown that AKR1C3 activation is a critical mechanism associated with enzalutamide resistance [53].

## 4. Conclusions

Despite promising advances in the treatment of metastatic prostate cancer, castration resistance remains a challenge for prostate cancer management. CRPC is characterized by multiple resistance mechanisms to ADT and remains an incurable disease. It has been shown that androgens and AR are crucial drivers of CRPC. AR-dependent mechanisms leading to resistance include AR amplification, AR mutations resulting in AR promiscuity, AR splice variants, and aberrant activation of AR (Fig. 1). New agents approved for treatment of CRPC such as enzalutamide and abiraterone acetate target a subset of these resistance mechanisms. However, mechanisms of resistance evolve over time against these new agents as well. It has been found that different agents such as niclosamide (an antihelminthic drug) and indomethacin (an AKR1C3 inhibitor) overcome resistance [51–53]. Further understanding of the mechanisms underlying resistance is necessary to identify future therapeutic targets.

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*Study concept and design:* Tilki, Evans.
*Acquisition of data:* Tilki, Evans.
*Analysis and interpretation of data:* Tilki, Evans.
*Drafting of the manuscript:* Tilki, Evans.
*Critical revision of the manuscript for important intellectual content:* Tilki, Schaeffer, Evans.
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*Supervision:* Tilki, Evans.
*Other:* None.

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