

## Molecular Basis of Drug Resistance and Insights for New Treatment Approaches in mCRPC

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**Abstract.** *Inhibiting androgen receptor (AR) signaling with androgen deprivation therapy (ADT) represents the mainstay of therapy for advanced and metastatic prostate cancer. However, about 20-60% of patients receiving first-line treatment for prostate cancer will relapse, evolving in a more aggressive and lethal form of the disease, the castration-resistant prostate cancer (CRPC), despite the use of ADT. Multiple approved systemic therapies able to prolong survival of patients with metastatic CRPC (mCRPC) exist, but almost invariably, patients treated with these drugs develop primary or acquired resistance. Multiple factors are involved in CRPC treatment resistance and elucidating the mechanisms of action of these factors is a key question and an active area of research. Due to such a complex scenario, treatment personalization is necessary to improve treatment effectiveness and reduce relapse rates in CRPC. In this review, current evidence about the major mechanisms of resistance to the available prostate cancer treatments were examined by introducing insights on new and future therapeutic approaches.*

Inhibiting androgen receptor (AR) signaling with androgen deprivation therapy (ADT) [*i.e.* surgical castration, luteinizing hormone-releasing hormone (LHRH) analogues] represents the first example of a 'targeted' therapy for advanced and

metastatic prostate cancer (PCa). About 20-60% of patients receiving a primary treatment for their PCa will relapse, despite use of ADT (1-3).

Tumor outgrowth during ADT represents a key transition point from hormone-sensitive disease to a more aggressive and ultimately lethal form, the castration-resistant prostate cancer (CRPC) (4). Currently, there are multiple approved systemic therapies able to prolong survival of patients with mCRPC. These include cytotoxic compounds (*i.e.* docetaxel and cabazitaxel), AR-targeted therapies (*i.e.* enzalutamide and abiraterone), and the radioisotope radium-223 (5-8). Unfortunately, most patients treated with these drugs develop primary or acquired resistance during their clinical course. Therefore, knowledge of resistance mechanisms to the available therapeutic agents for mCRPC is a key question and an active area of research (9)

Herein, current evidence about the major mechanisms of resistance to PCa treatments is reviewed and insights on new and future therapeutic approaches are introduced.

### Taxanes: Mechanism of Action

Taxanes represent an important chemotherapeutic class of drugs, which have demonstrated survival benefits in mCRPC therapy.

In 2004, docetaxel became the standard treatment for chemotherapy-naïve mCRPC patients, according to Phase-III clinical trials TAX327 and SWOG 99-16. These large trials compared patients treated with docetaxel *vs.* mitoxantrone and showed longer overall survival (OS) in docetaxel group (5, 6). Cabazitaxel, a new-generation taxane, was developed to overcome resistance to docetaxel, because of its low affinity for P-glycoprotein (P-gp), an ATP-dependent drug efflux pump. In TROPIC Phase-III trial, cabazitaxel plus prednisone therapy demonstrated a prolonged survival (up to 15.1 months) and a

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reduced risk of death (up to 30%) in mCRPC patients progressed during or after docetaxel-based therapy (6).

Microtubules are composed of a backbone of tubulin dimers and microtubule-associated proteins. The mechanism of action of taxanes can be explained by their capacity to bind strongly to the polymers of tubulin. Taxanes site of action is  $\beta$ -tubulin and their antimetabolic effects are exerted by reducing spindle microtubule dynamics, leading to the arrest of the cell cycle in the mitotic phase ( $G_2/M$ ). Taxanes have been reported to induce apoptosis, activation of several cellular signaling pathways, inhibition of intracellular trafficking, proteins nuclear translocation decrease (*i.e.* p53, AR) (10-14).

Despite numerous studies, the exact mechanisms of both taxane action and resistance are still unclear. The resistance is due to several contributing factors, such as: tubulin alterations, kinesins, overexpression of multi-drug-resistance (MDR), ETS fusion genes family, signaling pathways and cytokines and epithelial-mesenchymal transition (EMT), AR splice variants (15-17). Elucidating the mechanisms of taxane resistance may provide evidence for the discovery of novel biomarkers and drug development in CRPC.

### Tubulin Alteration

As far as taxanes target the cytoskeleton directly, several studies have demonstrated that a mechanism of resistance is performed by a different expression of  $\beta$ -tubulin subunits, which could influence the response to these drugs. In 2010, Terry *et al.* have reported an association between high expression of  $\beta$ III-tubulin and tumor aggressiveness in PCa patients receiving taxane-based chemotherapy. They also showed that  $\beta$ III-tubulin silencing increased sensitivity to docetaxel. According to their findings,  $\beta$ III-tubulin expression might be a predictive biomarker of tumor-resistance, mainly associated with local-spreading disease, involved lymph-nodes, and increasing prostate-specific antigen (PSA) levels (15).

Overall the results of the various conducted studies highlight the difficulty in isolating the contribution of single tubulin isotypes in drug response. Ranganathan *et al.* tested the effects of  $\beta$ -tubulin overexpression on anti-microtubule drug response, in human prostate carcinoma cells. They revealed that overexpression of  $\beta$ III isotype in human prostate carcinoma cells by stable transfection failed to confer anti-microtubule drug resistance to these cells. This finding emphasizes the difficulty in isolating the contribution of single tubulin isotypes in drug response studies (16).

Two further studies of the groups of Hari *et al.* and Hara *et al.* have shown that other potential mechanisms of tubulin-mediated resistance are the point mutations in  $\alpha$ - and  $\beta$ -tubulin. In their preclinical experiences,  $\beta$ -tubulin mutations, such as T26A, A595G, and F270I, have been related to an

impaired taxane-mediated polymerization. However, it is still unknown whether these mutations are simple epiphenomenon or an effective resistance mechanism, and their biological relevance, *in vivo* (17, 18).

Recently, new drugs have been tested in an attempt to overcome chemo-resistance. For example, Soucek *et al.* studied NanoOrl, a new formulation of the drug Orlistat (19). Taccalonolides, a new class of microtubule stabilization agents derived from plants of the genus *Tacca*, have been shown to overcome taxane resistance through stabilizing microtubules in a different manner compared to other microtubule stabilizers (20).

In conclusion, several new molecules have to be more investigated to identify biomarkers in CRPC.

### Kinesins

Kinesins, which are involved in intracellular microtubule transport and mitosis, have been studied regarding CRPC response to therapy. Sircar *et al.* demonstrated that the mitotic centromere-associated kinesin (MCAK) had increased expression in docetaxel-resistant CRPC compared to chemotherapy-naïve CRPC and hormone-sensitive PCa. Moreover, MCAK knockdown experiments resulted in growth arrest of prostate cancer cells, thereby suggesting MCAK as a novel target for CRPC therapy (21). On the other hand, another kinesin, Eg5, was significantly related to major docetaxel response rate, according to Wissing's study (22).

### Multidrug Resistance Phenotype

One of the most investigated features in taxane resistance mechanism in mCRPC is overexpression of ATP-binding cassette (ABC) or multidrug resistance (MDR) transporters (23). These proteins are transmembrane ATP-dependent efflux pumps, belonging to the ABC transporter superfamily, that have the ability to carry drugs and other xenomolecules out of the cells. Prostate tumor grade, stage, and PSA level have been directly correlated with expression of ABCB1; moreover, worse clinical outcome and toxicities have been associated with ABCB1 gene allelic variants (24).

Van Brussel *et al.* (25) studied the role of the MDR phenotype in several human PCa cell lines, and determined the expression of several proteins involved in this process, like Bcl-2, Bax, P-glycoprotein (Pgp), multidrug resistance-associated protein (MRP), topoisomerase (Topo) I, II $\alpha$  and II $\beta$ , glutathione-S-transferase- $\pi$  (GST- $\pi$ ). Based on their findings, Zhu *et al.* (26) studied the role of ABCB1 in docetaxel resistance. They found that overexpression of ABCB1 (P-glycoprotein/MDR1), ABCC4 and ABCC5 promoted taxane resistance, suggesting that blocking ABCB1 could represent a novel approach for resensitizing to docetaxel treatment. Furthermore, they showed (27) that

Table I. *Mechanisms of resistance to taxanes.*

Tubulin alterations	Hyper-expression of beta-III tubulin and mutations in alpha and beta tubulins increases taxane resistance
Kinesins	Kinesins protein family is implicated in taxanes- resistance mechanisms by interacting with microtubules
Multidrug Resistance Phenotype	Overexpression of ABAB1, ABCC4 and ABCC5 promotes taxanes resistance through increasing their excretion from tumoral cells
ERG and TMPRSS3-ERG Fusion Gene	When fused together ETS proteins are aberrantly transcribed, decreasing taxanes sensitivity
Growth Factors, Apoptosis Regulators and Intracellular Pathways	Reduction of apoptosis regulators, increasing of GATA2 and IGF2 growth factors and up-regulation of Notch and Hedgehog signalling pathways are linked to cancer resistance
EMT Transition	In Epithelial-to-Mesenchimal transition there is a reduced expression of micro-RNAs such as miR-200C and miR-205 associated with docetaxel resistance
Cytokines/Inflammation	IL-6, IL-8, chemokines ligand2, TGF beta1 and MIC-1 promote Docetaxel resistance
Androgen Receptor	Androgen Receptor splicing variants, such as ArV567 and ArV7 are implicated in taxanes sensitivity

bicalutamide and enzalutamide inhibited ABCB1 ATP-binding cassette transporter activity through blocking ABCB1 efflux activity by 40% and 60%, respectively, and in a dose-dependent manner. Bicalutamide, therefore, has been shown to resensitize cells to docetaxel treatment independently of the AR status, *in vitro* and *in vivo*; the association of bicalutamide with docetaxel was reported to have an important antitumor effect in AR-positive and AR-negative mouse models.

Oprea-Lager *et al.* (28) demonstrated that ABCC4, which is induced by androgens, is involved in docetaxel resistance. In their preclinical study, the authors observed that the ABCC4 protein (determined by western blotting) was expressed in taxane-resistant cells lines (MLL), while no ABCC4 was detectable in prostate cancer cells line (PCa3) sensitive to docetaxel and cabazitaxel. Also, they revealed that ABCC4 inhibition by the MRP inhibitor MK571 almost completely reversed resistance *in vitro* to docetaxel of MLL cells. Thus, cabazitaxel was found to be equally effective in both MLL and ABCC4 models, while ABCC4-positive cells line was far less sensitive to docetaxel (29). Cabazitaxel has therapeutic efficacy independently of ABCC4 expression.

The main taxane mechanisms of resistance are summarized in Table I.

### ERG and TMPRSS2-ERG Fusion Gene

Tomlins *et al.* (30) showed that gene fusion between TMPRSS2 (encoding a transmembrane serine protease constitutively expressed in prostate) and ETS family genes (ERG, FL11, ETV4 and ETV5) occur at a high frequency in PCa. When fused together, ETS proteins were aberrantly transcribed; the downstream activation of ETS target genes resulted in abnormal cellular proliferation. In another *in vitro* study, it was demonstrated that the TMPRSS2-ERG rearrangement might have a predictive value for response to Poly (ADP-ribose) polymerase (PARP) inhibitors in early and metastatic PCa (31).

ERG affects several parameters of microtubule dynamics and inhibits effective drug-target engagement of taxanes with tubulin (32). Galletti *et al.* studied the effects of ERG overexpression using *in vitro* and *in vivo* models of CRPC, and they revealed its capacity to decrease sensitivity to taxanes. They analysed a cohort of 34 men with mCRPC and showed that the over-expression of ERG doubles the probability of docetaxel resistance when compared with ERG-negative cancer. Specifically, they observed a PSA response to docetaxel therapy in the 45% and 79% of ERG-TMPRSS2 positive and negative patients respectively ( $p=0.056$ , odds ratio=0.23 (0.05-1.09 95% confidence interval).

It has been proven that ERG protein acts like a regulator of gene expression inside the nucleus, but in the cytoplasm, it can also bind to soluble tubulins, with detriment of the tubulin polymer, that are the main target of taxanes antitumoral action (33). This leads to a lack of sensitiveness towards these drugs but on the other hand to sensitization to microtubule depolymerizing drugs (34).

### Growth Factor, Apoptosis Regulators, and Intracellular Pathways

Several studies have demonstrated that docetaxel-resistant PCa cells show alterations of cell survival factors and apoptosis regulators such as Bcl-2, clusterin, heat shock proteins (HSPs), and activation of transcription pathways (STAT1, STAT3, NF- $\kappa$ B) (35). Other molecules and pathways associated with cancer chemotherapeutic resistance are p53, NF- $\kappa$ B, PI3K-Akt, and interleukin (IL)-6/STAT3 (36); some of these have also been related to resistance to taxanes (37-39). Vidal *et al.* (40) described 13 different genes that were frequently deregulated in docetaxel-resistant-PCa datasets. Among these genes, GATA2 and insulin-like growth factor 2 (IGF2) were increased in taxane-resistant cell lines and CRPC progression, and conferred aggressiveness in PCa cells. Overexpression of Notch and Hedgehog signaling pathway have also been shown to confer taxane resistance in

PCa (41). Finally, Liu *et al.* (42) demonstrated the association between p53 phosphorylation and docetaxel sensitivity in PCa cells.

### Epithelial-to-mesenchymal Transition (EMT)

Puhr *et al.* (43) demonstrated that expression of microRNA (miR)-200c and miR-205 in docetaxel-resistant cell lines (PC3-DR and DU-145-DR) which underwent EMT was significantly reduced compared to non-docetaxel resistant human prostate cell lines. Cells with the mesenchymal phenotype showed increased invasive and migratory abilities and decreased adhesive properties. Moreover, tumor relapse associated with reduced E-cadherin levels (44).

### Cytokines and Inflammation

There is evidence that overexpression of inflammation-associated molecules (*i.e.* pro-inflammatory cytokines) can lead to docetaxel resistance. Overexpression of IL-6 inhibits apoptosis and promotes cell proliferation. Furthermore, IL-8, transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), chemokine ligand 2 (CCL2) (45) and macrophage inhibitory cytokine-1 (MIC-1) are linked to docetaxel resistance. Mahon *et al.* (46) demonstrated that expression levels of several inflammatory cytokines were associated with docetaxel resistance *in vitro*. Other studies underline that increased CCL2 expression is associated with tumour progression, invasiveness, and poor prognosis. AR activation has been shown to enhance macrophage recruitment *via* CCL2 up-regulation (47).

### AR Receptor Variants in Taxane Sensitivity

Several works have suggested that the microtubule network in prostate cells is critical for AR signaling and nuclear translocation, where AR exerts its transcriptional activity. Docetaxel effectively targets the AR signaling axis in CRPC by blocking its nuclear translocation through microtubule stabilization. AR splice variants were found to be implicated in sensitivity to taxanes.

Thadani-Mulero *et al.* (12, 13) studied the two most clinically prevalent receptor splice variants, AR-V567 and AR-V7, presenting different taxane sensitivity, both *in vitro* and *in vivo*. It was reported that taxane treatment did not affect nuclear accumulation and transcriptional activity of AR-V7, whereas inhibited splice variant AR-V567. Molecular analysis demonstrated the lack of the hinge region in AR-V7, which might explain the differential interaction of the splice variants with the microtubule system. Zhang *et al.* (14) found that the expression of major variant AR-V7 was up-regulated in taxane-resistant cell lines. However, Onstenk and colleagues (48) investigated the response to cabazitaxel related to the AR-V7 status of CTCs from mCRPC patients.

According to their phase-II study, sensitivity to cabazitaxel was independent of AR-V7 variants. Thus, cabazitaxel might be a valid treatment option for AR-V7-positive patients.

Antonarakis *et al.* (49) studied clinical implication of AR-V7 variant status on taxane treatment outcome. In taxane-treated patients, no statistically significant differences were observed in PSA response, PSA progression-free survival (PFS), and median PFS according to AR-V7 status (AR-V7-positive *vs.* AR-V7-negative patients). However, in AR-V7-positive patients, when considering clinical outcomes, taxanes appeared superior to AR-directed therapy. This study revealed a higher PSA response, a longer PSA PFS and a better median PFS in mCRPC patients treated with taxane *vs.* AR-directed therapy.

Currently, ongoing prospective clinical trials aim to investigate AR-V7 status in taxane-treated mCRPC (50). In conclusion, more evidence is required to define the role of AR-V7 variant as a treatment-specific biomarker in CRPC.

### AR Mutation and Expression of Constitutively Active AR-variants

About 10-30% of CRPC patients reported an AR mutation (51). Known AR mutations include gene amplification, which leads to the overexpression of the receptor, point mutations, and constitutively active AR splice variants. AR point mutations in the regions coding for the ligand-binding domain (LBD) are implicated in drug resistance (52). These mutations may alter ligand binding and result in gain of AR function (53). AR point mutations are becoming more involved in the acquired resistance to abiraterone and next-generation AR antagonists (enzalutamide and apalutamide) (54). The AR mutation Phe876Leu results in the switch of ligand-binding specificity from agonist to antagonist and it has been associated with resistance to second-generation antiandrogens enzalutamide and ARN-509 (55).

Moreover, in abiraterone/prednisone-treated patients, the AR T878A and AR L702H mutations were also studied, which may facilitate the AR activation by progesterone or by glucocorticoids, respectively (56). It has been shown that patients harboring these activating AR mutations have worse survival compared to patients that harbor neither mutated nor amplified AR. The genome rearrangement and alternative splicing of splicing factors, such as hnRNPs (57), generate truncated versions of the wild type gene AR, called "AR splice variants", which lack the LBD but are often ligand-independent and constitutively active (58). The expression of AR splice variants has been implicated mostly in abiraterone and enzalutamide resistance. Amongst the 22 AR splice variants known, AR-V7 is the most expressed and studied. Antonarakis *et al.* published a prospective study on a cohort of 62 patients treated with enzalutamide or abiraterone, in which AR-V7 status was determined by a circulating tumor

cell (CTC)-based real-time polymerase chain reaction (PCR). They found an association between AR-V7 expression and clinical outcome. More specifically, both treatments with enzalutamide or abiraterone in patients with AR-V7 positive expression had significantly lower PSA response, shorter PFS and OS compared to AR-V7 negative patients (59). Although these results should be confirmed in larger prospective studies, they strongly suggest that patients with AR-V7 positive CTCs may have primary resistance to abiraterone and enzalutamide. As mentioned above, no changes in PSA response and PFS were observed in patients with high level of AR-V7, treated with docetaxel or cabazitaxel, suggesting that AR-V7-positive patients may be better candidates for taxanes as primary treatment (49, 50). Also, preliminary data on liquid biopsy for CTCs-based AR-V7 analysis showed that some patients may develop this aberration upon disease progression, as well as other AR-V7-positive patients may become AR-V7-negative after treatment with taxane-based chemotherapy. However, these data need to be confirmed.

### Non-genomic AR Resistance

Ligand-independent activation of AR may be heightened by the activated pathways of non-genomic AR signaling. Although AR action mainly occurs in the cell nucleus, where it regulates the expression of target genes (canonical signaling of AR), the initial androgen binding to AR LBD in the cytoplasm may already initiate signal transduction pathways capable of influencing cell proliferation and migration (non-genomic signaling of AR) (60). This pathway is generally androgen-dependent, but it can be also triggered by growth factors and their receptors in the absence of androgens (61). Bicalutamide and flutamide are not effective in blocking non-genomic activation of AR. The second-generation anti-androgens, such as enzalutamide, apalutamide (ARN-509), and darolutamide (ODM-201), show higher affinity for the AR LBD and could minimize nuclear translocation of full-length AR, resulting in the AR cytosol accumulation (62, 63). Cytosolic AR could interact with Src3 and PI3K proteins activating their intracellular signaling pathways and enhancing cell proliferation and survival. In presence of low androgen levels, as occurred during an antiandrogen therapy, the proline-rich sequence between residues 371 and 381 in the N-terminal domain (NTD) of AR interacts with Src homology domain 3 (SH3), activating prominent intracellular signaling pathways to enhance cell proliferation and survival. Indeed, the release of epidermal growth factor (EGF) or IL-6 as well as MAPKs, phosphoinositide 3-kinase (PI3K)/Akt and protein kinase A pathways were implicated in the stimulation of ligand-independent AR activity by a non-genomic mechanism.

Meanwhile, the expression of constitutively active AR splice variants, such as AR-V567es and V7, localized in the nuclei, do not mediate non-genomic signaling. Other splice

variants have been discovered to have non-genomic actions, mainly AR8 and AR23. AR8 is endogenously expressed in several CRPC cell lines (C4-2B, 22Rv1, and CWR-R1) and is responsible of androgen-independent growth. AR23 is a cytosolic AR variant able to potentiate transcription, mediated by full-length AR and influence transactivation of NF- $\kappa$ B in reporter gene assays (64).

### New Treatment Strategies Focus on Non-genomic Resistance

A new class of drugs that inhibit the AR NTD interaction with Src kinase may be considered to block non-genomic signals from full-length AR, or possibly membrane-associated or cytosolic AR splice variants, in order to prevent tumor growth. *In vitro* studies have shown that it is possible to block the growth of prostate tumor cell lines by blocking the AR/Src3 interaction through the use of proteins that mimic or eliminate the AR polyproline sequence (65). Non-genomic effects of AR might be also prevented by destroying AR protein or by non-AR-targeting compounds, such as galeterone (TOK-001) and niclosamide, discussed in the paragraph below.

### New Treatment Strategies Focus on Suppressing AR Activity

Several ongoing trials aim at examining the efficacy of novel drugs (ARN-509 and ODM-201) targeting AR splice variants, in order to overcome mechanisms of resistance to the novel AR targeting molecules (abiraterone acetate and MDV-3100) (66-67). ARN-509, a next-generation AR antagonist, showed clinical benefit in mCRPC patients with or without prior abiraterone acetate/prednisone treatment, with a good toxicity profile (67). Darolutamide (ODM-201), is a full AR antagonist with very high-affinity to LBD and able to inhibit AR nuclear translocation. ODM-201 may also antagonize the F876L mutated AR, which was detected and probably involved in some cases of acquired resistance to ARN-509 and ENZ. In phase I/II clinical trial ODM-201 showed a promising anticancer activity in both chemotherapy-naïve and pre-treated mCRPC patients, but significantly lower in patients previously treated with CYP17 inhibitors (66). Thus, it was hypothesized that ODM-201 could be used in case of ENZ, ARN-509, bicalutamide, and flutamide resistance. Therefore, a phase III trial to assess the efficacy of ODM-201 plus standard ADT plus docetaxel (6 cycles after randomization) in men with metastatic hormone-sensitive prostate cancer is ongoing (NCT02799602).

A desirable feature of AR NTD antagonists is their ability to inhibit full length-AR and AR splice variants, regardless of AR LBD. Two families of compounds are involved in binding the AR NTD and inhibiting transactivation: sintokamide A and EPI compounds (68-69). In particular,

both these agents have shown promising results in overcoming the resistance to enzalutamide in CRPC xenografts, in mice. EPI is a drug that covalently binds the NTD of both AR and AR variants, inhibiting their transcriptional activity. *In vivo* administration of EPI in PCa xenograft models was observed to reduce tumor growth. EPI-506 (ralaniten acetate) is the first AR NTD antagonist to be tested in clinical trials for mCRPC (NCT02606123). Recently, the safety results of the open-label, single-arm, phase 1/2 study testing EPI-506 mCRPC patients progressed after  $\geq 1$  line of hormonal therapy or chemotherapy and progressed on enzalutamide and/or abiraterone have been presented during 2017 ASCO meeting. Data on the eighteen patients enrolled showed that EPI-506 is well-tolerated (70).

Identification of agents able to overcome mechanism of resistance by inhibiting the AR outside of LBD may be an effective strategy to reintroduce drug sensitivity and decrease CRPC tumor progression (71).

Niclosamide, recently approved by the Food and Drug Administration (FDA), is an anti-helminthic drug with a known AR-V7 inhibitory activity. Niclosamide induced both degradation of AR-V7 protein and genes transcriptional activity (72). Moreover, niclosamide improves enzalutamide sensitivity by modulating Stat3 activity. In particular, this drug is able to inhibit Stat3 phosphorylation, to reduce target gene expression, and to abrogate recruitment of AR to the PSA promoter (73). Another study by Nadiminty *et al.* showed that CRPC cells resistant to enzalutamide were resensitized by down-regulating the splicing factor hnRNP1, which was implicated in the AR-V7 expression (57).

Galeterone (TOK-001) is a novel agent under investigation that could potentially target full length and AR-V7 splice variants (74). It acts both as an inhibitor of the enzyme CYP17A1 and as a direct AR antagonist, that has been shown to prevent AR binding to chromatin and enhance AR mutant degradation in PCa cell lines (75).

ASC-J9, a molecule studied in xenograft tumor models, showed the ability to decrease prostate tumor growth by degrading both full length and AR-V3 splice variants (76).

Finally, niphatenones are another class of drugs that target the NTD; however, alkylation reaction test using glutathione revealed that niphatenone B enantiomers are random alkylators forming adducts with glutathione. Therefore, niphatenones as non-selective alkylators due to potential haptenization and toxicity were not recommended as promising candidates for PCa therapy (77).

## The Future of Targeted Therapies and Precision Cancer Care

"Precision medicine" is a novel approach for personalized disease management based on specific genetic and environmental biomarkers for each patient. Thus, biomarkers

providing patient- or tumor-specific information are critical in precision medicine applied to oncology (78).

In PCa, there are prognostic biomarkers which have been variously associated with different end-points including: PSA level, lactic dehydrogenase (LDH), alkaline phosphatase and hemoglobin. Many other potential biomarkers also exist, such as: CTCs count (79, 80), DNA aberrations, and RNA transcriptional profiling (81). Biomarkers are ideally able to predict sensitivity or resistance to a particular therapy. As already mentioned, much attention has been given to AR-V7 in CTC RNA assays, as a predictive biomarker of resistance to abiraterone and enzalutamide. AR gene, located on Xq12 consists of eight exons and many other DNA regions with regulatory activity. AR gene splicing may contribute to the development of truncated AR variants which are more frequent in CRPC than in hormone-naïve PCa patients. Specifically, the AR-V7 is devoid of LBD for androgens. The presence of AR-V7 protein in CTCs has been associated with resistance to AR-directed therapy, but not to taxanes (49, 59).

Furthermore, mCRPCs show an extensive mutational landscape, which indicates the possibility of targeted therapies and precision medicine. The use of genomic profiling of single tumors is rapidly changing treatment strategies across different cancer types, including mCRPC.

Recent reports based on the use of integrative whole-exome sequencing (WES) and whole transcriptome sequencing in metastatic site (bone or soft tissue) biopsies from 150 mCRPC patients have shown that about 90% of mCRPC harbor a potentially actionable molecular alteration including: 63% aberrations in AR (enriched in mCRPC compared to primary PCa), 65% aberrations in other cancer-related genes (*e.g.* ETS, TP53, PTEN), 23% aberrations of DNA repair pathway (BRCA1, BRCA2, ATM), and 8% pathogenic germ-line alterations and epigenomic alterations (DNA methylation, HDAC) (82).

Manish Kohli *et al.* have more recently shown results of their whole-exome/RNA-sequencing analysis in metastatic biopsies from 92 CRPC patients treated with abiraterone acetate plus prednisone. The gene analysis of non-responder patients (progression at 12-weeks of therapy) revealed frequent mutations in Wnt/beta-catenin pathway genes and frequent deletion of negative regulators of Wnt pathway (DKK4, SFRP2, and LRP6). The authors also revealed a different expression level of Wnt/beta catenin pathway inhibitors and cell cycle proliferation genes in non-responders (83).

Furthermore, recent evidence from a study on molecular signature in PCa has suggested the possibility to identify the "aggressive variant of PCa" (AVPC), a morphologically heterogeneous PCa which clinically mimics the behaviour of small cell PCa. Authors showed that AVPC can be diagnosed based on a distinct molecular signature (MS), defined as combined tumor suppression defects of  $\geq 2$  alterations of tp53, RB1 and/or PTEN by immunohistochemistry (IHC) or

Table II. Principal clinical trials in advanced castration-resistant prostate cancer.

	Gene	Potential therapeutic	Current clinical trials/therapies	Phase
Androgen receptor	Androgen receptor	Mifepristone (RU-486)	NCT00140478	Phase 2
		Mifepristone/enzalutamide	NCT02012296	Phases 1, 2
		Galeterone	NCT02438007	Phase 3
		GTx-758	NCT01615120	Phase 2
		VT-464	NCT02445976	Phase 2
		Trilostane	NCT00181597	Phase 2
		AZD3514	NCT01162395	Phase 1
		Orteronel (TAK-700)	NCT00569153, NCT01809691,	Phases 1, 2, 3
			NCT01809691	
		Triamcinalone	NCT00186108	Phase 1
Immunotherapy		Ipilimumab + ADT	NCT01377389	Phase 2
		AR DNA Vaccine	NCT02411786	Phase 1
		Ipilimumab	NCT00170157	Phase 2
		BNIT-PR-001		Phase 1
PIK3CA	PIK3CA	Buparlisib (BKM120)	NCT02487823	Phase 1
	PIK3CB	AZD8186	NCT01884285	Phase 1
	PIK3CB	GSK2636771/enzalutamide	NCT02215096	Phase 1
	PIK3CB	GDC-0068/abiraterone	NCT01485861	Phase 2
Cell Cycle	BCL-2	Navitoclax/abiraterone	NCT01828476	Phase 2
	CDK4/6	Ribociclib	NCT02555189	Phases 1, 2
	CDK4/6	PD 0332991	NCT02059213	Phase 2
DNA damage	PARP	Niraparib/enzalutamide	NCT02500901, NCT00749502	Phase 1
		Olaparib/enzalutamide	NCT01972217	Phase 2
		BMN 673	NCT01286987	Phase 1
		Veliparib	NCT00892736	Phase 1
WNT	WNT	Foxy-5	NCT02020291	Phase 1
		OMP-54F28	NCT01608867	Phase 1

ADT, Androgen deprivation therapy; AR, androgen receptor; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; BCL-2, B-cell lymphoma 2; CDK4/6, cyclin-dependent kinase 4 and 6; PARP, poly (ADP-ribose) polymerase.

genomic analysis (84). They also studied AVPC-MS (by IHC) in mCRPC patients treated with taxane alone or with platinum. In 160 mCRPC patients enrolled in the phase 2 clinical trial comparing cabazitaxel (CAB) to CAB plus carboplatin (CARB), it was found that AVPC-MS (by IHC) patients had longer mPFS when they received CARB in addition to CAB. Thus, AVPC-MS was suggested as a potential predictor for platinum benefit. Results from genomic profiling of DNA samples are pending (85).

In conclusion of the aforementioned, the AR and its signaling pathway remain the principal drivers of development and progression of PCa, even in the advanced stages of disease (like CRPC). For this reason, they remain the main targets of the principal therapeutic approaches for PCa. A key question for the choice among the different available drug options is how to identify patients that will most benefit from their use.

Validated biomarkers in precision cancer care may be used for the selection of targeted therapies (when the biomarker is the target itself) or for the identification of patients more responsive to the standard therapies. Many ongoing clinical

trials (Table II) explore the potential precision medicine applications in advanced CRPC and may respond to these questions.

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