

Resistance to Novel Antiandrogen Therapies in Metastatic Castration-Resistant Prostate Cancer

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Supplementary Issue: Key Difficulties Associated with Cancer Biology

ABSTRACT: Despite the introduction of novel therapies that maximally decrease androgen-receptor (AR) signaling activity, metastatic castration-resistant prostate cancer (mCRPC) remains a lethal disease. Even though abiraterone and enzalutamide represent breakthroughs in the treatment of mCRPC and have demonstrated significant survival benefits, a significant proportion of patients have primary resistance to these agents and virtually all patients develop secondary resistance. While the mechanisms of resistance to these agents are not fully understood, many hypotheses of AR-dependent and AR-independent mechanisms are emerging, including upregulation of AR and cytochrome P450 17 α -hydroxylase/17,20-lyase (CYP17), induction of AR splice variants, AR point mutations, upregulation of glucocorticoid receptor, activation of alternative oncogenic signaling pathways, neuroendocrine transformation, and immune evasion via programmed death-ligand 1 upregulation. The aim of this review is to summarize the most clinically relevant mechanisms of resistance to novel androgen-directed agents, focusing on escape from enzalutamide and abiraterone.

KEYWORDS: castration-resistant prostate cancer, novel androgen-directed therapy, resistance, abiraterone, enzalutamide

SUPPLEMENT: Key Difficulties Associated with Cancer Biology

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Introduction

Metastatic castration-resistant prostate cancer (mCRPC) is the final common pathway in the disease continuum of prostate cancer and remains a lethal phenotype that leads to a significant burden of morbidity and mortality worldwide. In the US, prostate cancer is the second most common cause of cancer deaths in men, and approximately one in every six American men will be diagnosed with this disease during his lifetime.¹ Most patients who eventually develop mCRPC are initially diagnosed with localized high-risk disease that progresses after treatment. Less than one-third of patients are diagnosed with metastatic prostate cancer at disease presentation. While mCRPC currently benefits from a wealth of treatment options, the disease remains incurable. It is becoming increasingly understood that this disease entity continues to evolve over time, acquiring additional and diverse resistance mechanisms with each subsequent therapy used.

It is now accepted that mCRPC is not androgen independent and continues to rely on androgen signaling, despite systemic androgen depletion strategies.² Owing to this new understanding, several novel drugs have emerged for the

treatment of mCRPC; these agents either suppress the synthesis of extragonadal androgens or target the androgen receptor (AR) directly.³ Abiraterone is an inhibitor of cytochrome P450 17A1 (CYP17A1) that impairs AR signaling by inhibiting both the 17 α -hydroxylase and 17,20-lyase activities of the CYP17A1 enzyme, thereby depleting adrenal and intratumoral androgen.^{4,5} Enzalutamide is an inhibitor of AR signaling that exerts its activity by binding avidly to the ligand-binding domain (LBD) of the AR, competing with and displacing the natural ligands of this receptor (testosterone and dihydrotestosterone), while also inhibiting translocation of the AR into the nucleus and impairing transcriptional activation of androgen-responsive target genes.^{6,7} After several studies showed augmented survival with these drugs,^{3,8,9} both agents were approved by the Food and Drug Administration for the treatment of mCRPC.

Although enzalutamide and abiraterone arguably represent significant advances in the treatment of mCRPC, approximately 20%–40% of patients have primary resistance to these agents and exhibit no response with respect to prostate-specific antigen (PSA) levels or other measures of

clinical benefit.^{3,7-9} Furthermore, among patients who initially have a serological or clinical response to enzalutamide or abiraterone, virtually all eventually acquire secondary resistance over time. While our understanding of prostate cancer biology and disease resistance mechanisms has grown over the past few years, drug resistance represents the primary challenge of treating prostate cancer today.

The aim of this review is to summarize the most clinically relevant mechanisms of resistance to novel androgen-directed agents (Fig. 1), focusing on enzalutamide and abiraterone. The diversity and heterogeneity of pathways in prostate cancer demands a critical consideration of the numerous biological events involved. Readers are referred to other reviews for a more comprehensive discussion of resistance to first-line hormonal therapies.^{10,11}

AR and CYP17 Upregulation

Resistance mechanisms leading to CRPC have been described affecting multiple parts of the AR axis. AR gene amplification and protein overexpression have been frequently observed in response to treatment with androgen deprivation therapy (ADT) and likely also play a key role in the development of resistance to novel antiandrogens such as abiraterone and enzalutamide. Indeed, over 80% of CRPC shows high levels of AR expression due to gene amplification and/or mRNA/protein overexpression.¹² Mostaghel et al demonstrated that treatment of human CRPC xenografts with abiraterone increased the expression of both full-length AR (FL-AR) and truncated AR splice variants by threefold.¹³ Additionally, Yamamoto et al showed that enzalutamide-resistant LNCaP cells express high levels of both FL-AR and AR splice variants

compared with CRPC LNCaP cells.¹⁴ The authors also showed that knockdown of AR (either FL-AR alone or FL-AR plus AR variants) suppressed cell growth and AR-regulated gene expression and delayed tumor growth *in vivo*. Therefore, AR appears to be one key driver in the enzalutamide-resistant LNCaP model.¹⁴ Several other studies have shown that AR amplification and AR gene aberrations in circulating cell-free DNA are associated with resistance to enzalutamide and abiraterone in mCRPC.^{15,16}

In addition, CYP17 upregulation (or upregulation of other androgen-synthetic enzymes) also appears to play a role in resistance to novel antiandrogens. Mostaghel et al showed that treatment with abiraterone increased expression of the *CYP17A1* gene twofold in relapsed tumors in the castration-resistant VCaP xenografts.¹³ It has also been reported that AR activity in CRPC xenografts is driven by CYP17A1-dependent de novo intratumor androgen synthesis.¹⁷ Salvi et al showed that circulating cell-free AR and *CYP17A1* copy number variations are associated with poor outcomes in patients treated with abiraterone.¹⁸ Furthermore, several other preclinical studies have shown that tumor relapse on abiraterone was associated with upregulation of intratumoral CYP17A1.¹⁹

AR Splice Variants

The discovery of alternative mRNA splicing variants of the AR in 2008²⁰ has added further complexity to our understanding of the role of androgen/AR signaling in mCRPC and is one putative mechanism for the resistance to both enzalutamide and abiraterone.^{13,21} At least 22 AR splice variants have been reported in the literature to date,^{22,23} with AR-V7 and AR^{v567es}

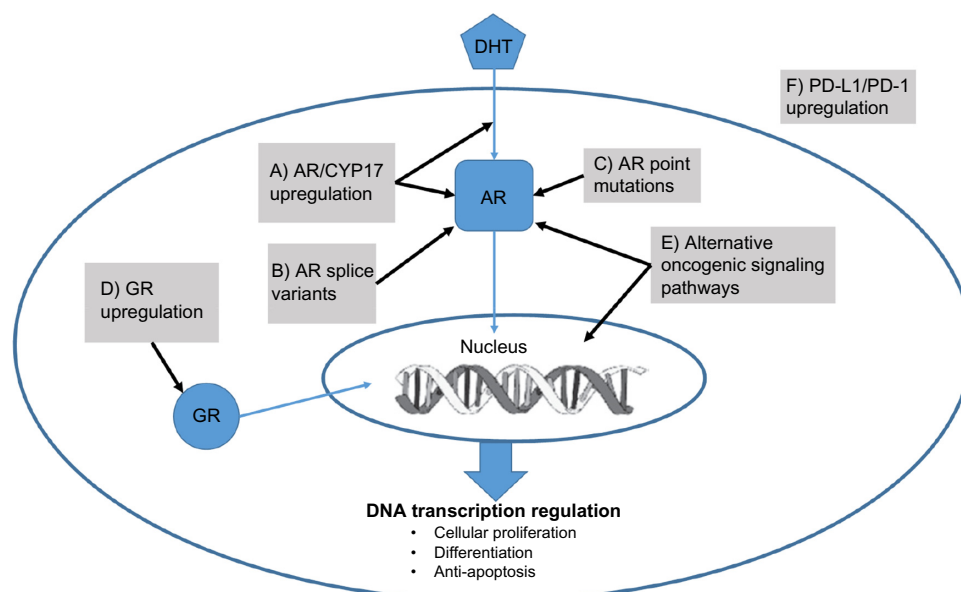


Figure 1. Mechanisms of resistance to novel antiandrogens: (A) AR and CYP17 upregulation, (B) AR splice variants, (C) AR point mutations, (D) GR upregulation, (E) alternative oncogenic signaling pathways, and (F) PD-L1/PD-1 upregulation. AR, androgen receptor; DHT, dihydrotestosterone; GR, glucocorticoid receptor.



being the most widely studied and perhaps the most clinically relevant. AR splice variants develop from alternative mRNA splicing events or (more rarely) through gene alterations, frequently in the noncoding regions of the AR gene, for example, by homologous recombination-independent mechanisms.²⁴ These alternative mRNA species encode a truncated AR protein that lacks the C-terminal LBD but retains the transactivating N-terminal domain.^{20,25} Although the resultant truncated proteins are unable to bind ligand, they are constitutively active as transcription factors and capable of promoting activation of target genes in a ligand-independent fashion. It has been repeatedly demonstrated that the expression of splice variants such as AR^{V567es} and AR-V7 is upregulated or induced by inhibition of the AR pathway with hormonal agents, such as abiraterone and enzalutamide.^{13,21,26} Mostaghel et al studied mRNA expression levels of AR-V7, FL-AR, and AR^{V567es} in a mouse xenograft model using LuCaP cell lines engineered to express AR-V7 and AR^{V567es}. Mice treated with abiraterone had significant improvement in median overall survival (OS) compared with placebo. At the time of disease progression, mRNA levels of FL-AR, AR^{V567es}, and AR-V7 were analyzed from harvested tumor tissues and were noted to be elevated by 3.4-fold ($P = 0.001$), 5.2-fold ($P = 0.073$), and 3.1-fold ($P < 0.001$), respectively, compared with baseline levels. Similar findings of higher mRNA expression levels of AR splice variants have been demonstrated in enzalutamide-resistant tumors in mouse xenograft models.^{21,26} For example, Nadiminty et al showed that NF- κ B2/p52 promotes resistance to enzalutamide in LNCaP-C42B and CWR-22Rv1 cell lines through upregulation of AR variants and that knockdown of FL-AR and AR-V7 increased the sensitivity of these two cell lines to enzalutamide.²¹ This higher expression in the setting of hormonal therapy suggests that splice variants are a clinically meaningful mechanism of drug resistance.

The first prospective clinical study reporting on the prognostic value of AR-V7 in the context of novel antiandrogen therapy was published by Antonarakis et al in 2014.²⁷ In that study, men with mCRPC embarking on treatment with standard-of-care abiraterone or enzalutamide were evaluated for AR-V7 mRNA expression in their circulating tumor cells (CTCs) using the AdnaTest platform (Qiagen). PSA responses (PSA decline $\geq 50\%$), progression-free survival (PFS), and OS were compared between patients positive for AR-V7 and patients without AR-V7 expression. Eighteen of the 62 patients (29%) tested positive for AR-V7 at baseline (12 of 31 enzalutamide-treated patients and 6 of 31 abiraterone-treated patients). None of these 18 AR-V7-positive patients had a PSA response with enzalutamide or abiraterone, compared with AR-V7-negative patients who had a 53% and 68% PSA response rate to enzalutamide and abiraterone, respectively. PFS was also markedly different between the AR-V7-positive and -negative cohorts at 2.1 and 6.1 months, respectively, for enzalutamide, and 2.3 and 6.3 months, respectively, for abiraterone. OS was also

decreased for the AR-V7-positive population in this study, with a median survival of 5.5 months vs not reached for the enzalutamide-treated patients in AR-V7-positive and -negative patients, respectively. In the abiraterone-treated patients, the median OS was 10.6 months vs not reached for AR-V7-positive and -negative patients, respectively. Interestingly, all of the patients with detectable CTC-derived AR-V7 at baseline remained positive for AR-V7 at the time of progression, while six patients (14%) who were AR-V7-negative at baseline converted to AR-V7-positive during the course of therapy with enzalutamide or abiraterone. These patients who converted had worse clinical outcomes compared with the patients who remained AR-V7 negative throughout the treatment with enzalutamide or abiraterone. This initial study provided preliminary evidence that AR-V7 detection in CTCs might be associated with primary resistance to novel antiandrogen therapies and suggested that expression of alternative AR splice variants is increased as a consequence of continued androgen-directed therapies.

More recently, Steinestel et al reported on their experience with AR-V7 detection and novel antiandrogen therapy resistance.²⁸ Patients with detectable CTCs using the AdnaTest platform (Qiagen) were evaluated for treatment response to abiraterone and enzalutamide. Overall, 18 of 37 patients (48%) were found to express AR-V7. They also noted an increased incidence of AR-V7 expression in patients receiving multiple prior AR-directed therapies compared with patients progressing after ADT alone (80% vs 28.6%, respectively). Ten of 14 AR-V7-negative patients (71%) demonstrated a PSA response to therapy, whereas only 1 of 15 AR-V7-positive patients (7%) had a PSA response to therapy, in this case to abiraterone.²⁸ While this report corroborates the study by Antonarakis et al, it also suggests the possibility of occasional responses to abiraterone/enzalutamide, despite tumoral expression of AR-V7.

Finally, Efsthathiou et al also evaluated 60 men with mCRPC for AR-V7 expression at the protein level from bone marrow biopsies prior to treatment with enzalutamide and after eight weeks of therapy.²⁹ Six of 12 (50%) patients with baseline AR-V7 protein expression using an immunohistochemical assay demonstrated primary drug resistance (ie, no PSA response to enzalutamide therapy), and none experienced benefit from enzalutamide treatment lasting longer than six months. The incidence of AR-V7 expression was 50% at baseline and increased to 70% after eight weeks of therapy in patients experiencing primary resistance to enzalutamide. Overall, the incidence of AR-V7 expression increased from 26% at baseline to 40% by the conclusion of the study in all 60 patients.²⁹ Given the high incidence of the splice variant expression noted in this study, AR-V7 appears to be a frequent cause of drug resistance to enzalutamide in this setting, although the differences between the CTC-derived mRNA-based assay and the bone marrow-derived protein-based assay should also be noted.



Although the discovery of the AR-V7 splice variant represents a significant advance in helping to elucidate the mechanisms of resistance to novel hormonal therapy, it is important to note the limitations of using AR-V7 testing in clinical decision-making. First, the presence of AR-V7 may merely represent a marker of aggressive disease rather than being a driver of therapeutic resistance. Furthermore, the clinical relevance of AR-V7 differs between treatment-naïve and heavily pretreated CRPC patients. Indeed, most patients included in the aforementioned AR-V7 trials are CRPC patients with advanced disease, who have progressed through multiple lines of hormonal therapy. Furthermore, AR-V7 testing is currently performed via a CTC-based assay that requires the presence of CTCs, so determination of AR-V7 status is not possible in patients without CTCs. Finally, AR-V7 testing is not currently commercially available outside of a Clinical Laboratory Improvement Amendments-certified laboratory at Johns Hopkins Hospital, Baltimore, MD, and, therefore, not broadly applicable outside of academic institutions.

AR Point Mutations

While exceedingly uncommon in primary hormone-sensitive disease, point mutations in the *AR* gene have been reported to occur at a comparatively high incidence (>10%) in patients with CRPC, especially in tumors progressing under systemic hormonal therapy and novel antiandrogens.^{12,30–32} As described by Grasso et al, the *AR* gene is among the nine genes that are most significantly mutated in mCRPC.³³ Mutations in the *AR* may have various effects, including loss of function, increased or decreased AR signaling, and some cause no change, while most mutations result in loss of function when studied in cell culture models.³⁴ In addition, these *AR* point mutations appear to be somatic events, with most being located in the LBD.³⁵ Gain-of-function mutations result in nonspecific activation of the AR-LBD by weak androgens, progestins, glucocorticoids, estrogens, and even antiandrogens.

One of the most frequently observed mutations is the T878A mutation (previously T877A).³⁶ This mutation appears to arise in the setting of treatment with androgen synthesis inhibitors such as abiraterone. Through CYP17 inhibition, the production of dehydroepiandrosterone and testosterone is suppressed, while upstream progesterone production is increased. This increased progesterone concentration may select for the T878A mutation. This mutant broadens the ligand-binding specificity of AR, sensitizing it to steroid hormones such as progesterone and estrogens.^{37,38} Interestingly, it also appears to sensitize the AR to some antiandrogens that are converted to strong agonists.³⁹ A recent study by Chen et al evaluated metastatic tumor tissue biopsy samples using targeted sequencing of the *AR* gene in 18 patients with mCRPC who progressed on CYP17 inhibitors (17 patients on abiraterone and 1 on ketoconazole).⁴⁰ Three of the 18 patients (17%) expressed the T878A mutation at a high allele frequency. Of note, this mutation does not appear to cause resistance to all antiandrogens,

as bicalutamide paradoxically demonstrated retained efficacy in one patient identified with a T878A mutation who previously progressed on flutamide, as reported by Joyce et al.⁴¹; the exact mechanism for this phenomenon remains unclear.

Another important mutation that has frequently been reported is the F877L mutation (previously F876L). This mutation has been demonstrated in both cell line models^{42,43} and patient tumor samples⁴⁴ and appears to arise in the setting of enzalutamide and ARN-509 (apalutamide) therapy. This mutation seems to sensitize the AR to enzalutamide and converts this agent from an antagonist into a strong agonist. In a study by Korpál et al, enzalutamide-resistant clones were created spontaneously by culturing LNCaP cells with enzalutamide for an extended period of time, and all resistant clones were found to express the AR F877L mutation.⁴³ This mutation has also been demonstrated clinically. A study of 62 patients progressing on abiraterone, enzalutamide, or other hormonal therapies identified missense *AR* mutations in exon 8 (the location of the LBD) in 11 (18%) patients, including the F877L mutation in two patients progressing on enzalutamide.¹⁵ This study also demonstrated the F877L mutation in patients progressing on ARN-509, a next-generation antiandrogen, which is structurally very similar to enzalutamide. Another study by Joseph et al found that enzalutamide and ARN-509 confer agonistic activity in F877L-engineered LNCaP cell lines.⁴⁴ The authors also tested circulating tumor DNA from plasma samples in patients treated with ARN-509 and found that 3 of 29 patients (10%) had induced expression of F877L after treatment with ARN-509. Of note, the F877L mutation does not appear to confer resistance to all AR antagonists. Korpál et al found that the ARF877L cells retained sensitivity to bicalutamide, despite resistance to enzalutamide in their *in vitro* assays⁴³; the reason for this remains unclear but suggests that the conformational change in the AR-LBD induced by the F877L mutation remains compatible with binding of bicalutamide in an inhibitory capacity.

Other *AR* point mutations that result in glucocorticoid activation of the AR have also been described.^{45,46} For example, the L702H mutation has been reported in patients taking abiraterone together with dexamethasone or prednisone.⁴⁷ In a study by Carreira et al, expression of the L702H mutation prior to treatment with abiraterone appeared to be associated with primary refractory disease to subsequent treatment with abiraterone. The authors also used an *in vitro* AR luciferase reporter assay to show that L702H was not inhibited by enzalutamide and was activated by prednisolone. Another study by Romanel et al demonstrated the emergence of L702H and T878A *AR* point mutations in 13% of tumors at progression on abiraterone.¹⁶ Overall, these results suggest that, similar to mutations in other therapeutically targeted oncogenes, *AR* point mutations sometimes provide a survival advantage to prostate cancer cells and promote resistance to novel antiandrogens such as abiraterone and enzalutamide.



Glucocorticoid Receptor Upregulation

Another potential mechanism of resistance by which tumors bypass AR blockade is through upregulation or induction of the glucocorticoid receptor (GR). The AR and GR belong to class I nuclear steroid receptors, which also includes the estrogen receptor and progesterone receptor. These receptors share many commonalities in their structure and mechanism of action. Indeed, the GR may be able to bind to AR promoter elements, thereby substituting for the AR in certain circumstances.⁴⁵ The AR and GR appear to have overlapping sets of gene targets and transcriptomes, suggesting that GR may be implicated in the development of resistance to antiandrogen therapy.⁴⁸ In a recent seminal study by Arora et al, LNCaP xenografts expressing wild-type AR were treated with novel AR inhibitors, including enzalutamide and ARN-509.⁴⁵ The authors found that many common gene targets of AR and GR were upregulated in the resistant tumors, while GR mRNA and protein levels were significantly higher in tumors resistant to enzalutamide and ARN-509. Furthermore, knockdown of GR in cells derived from resistant tumors restored the sensitivity to enzalutamide when administered in VCaP cells. Additionally, AR inhibition led to strong GR expression in a subset of prostate cancer cells in this study.⁴⁵ These findings support the hypothesis that GR upregulation promotes resistance to novel antiandrogens. The authors also evaluated the expression of GR in metastatic prostate cancer biopsies obtained from patients treated at MD Anderson Cancer Center, Houston, TX, with enzalutamide for eight weeks and showed that poor responders had higher levels of GR compared with good responders at the same time point and compared with baseline levels. This result was consistent with a previous study by Davies and Rushmere, which showed that GR expression in the ventral rat prostate increased after castration.⁴⁹ These conclusions highlight the potential role of GR in the development of resistance to novel antiandrogens.

Other Oncogenic Signaling Pathways

Alternative oncogenic signaling has also been implicated in the posttranscriptional activation of AR and development of treatment resistance. Indeed, androgen deprivation and AR inhibition lead to the activation of numerous oncogenic signaling pathways that promote the transcriptional activities of AR and induce prostate cancer cell growth. For example, Ueda et al found that steroid receptor coactivator-1 (Src-1) and interleukin-6 (IL-6) promote AR activation in the absence of androgens, while inhibition of mitogen-activated protein kinase abolished this effect.⁵⁰ Furthermore, IL-6 has been shown to promote resistance to bicalutamide through upregulation of the AR coactivator transcription intermediary factor 2 (TIF2).⁵¹

Human epidermal growth factor receptor 2 (HER2) and HER3 downstream signaling has also been associated with increased AR activity during prostate cancer progression.⁵² For instance, Mellinghoff et al found that knockdown

of HER2 inhibits AR transcriptional activity in LNCaP and LAPC4 cell lines, while HER2 and HER3 stabilize AR and increase its binding to androgen-responsive elements in the promoters of AR-regulated genes.⁵³ Furthermore, Chen et al found that androgen depletion increases HER2, thereby promoting AR stabilization and PSA production.⁵⁴

Activation of the phosphatidylinositol 3-kinase-Akt (PI3K-Akt) signaling pathway and loss of the phosphatase and tensin homolog (PTEN) tumor suppressor gene have also been shown to occur very frequently in mCRPC.³² Mouse models and cell line studies using LNCaP cells have shown that alterations in PI3K-Akt and PTEN activity using either targeted drugs or gene knockout techniques demonstrate changes to AR expression and AR transcriptional activity.^{55–57} Akt-mediated AR phosphorylation has been shown to increase the interaction of AR with the transcriptional factor p300/CBP, inhibiting AR ubiquitination and degradation.⁵⁸ Furthermore, high expression of p300 has been correlated with higher AR protein levels in human prostate cancer specimens and appears to be important for the androgen-dependent and androgen-independent transactivation of AR.^{58,59} Finally, PTEN deletion and subsequent Akt activation were also found to decrease AR protein levels and transcriptional activity through HER3 signaling alterations.⁵⁵

In addition, the signal transducer and activator of transcription 3 (STAT3) has been implicated in the development of mCRPC.⁶⁰ A recent study showed that AR inhibition and androgen depletion induces STAT3 activation and promotes the development of prostate cancer stem-like cells.⁶¹ Furthermore, Liu et al demonstrated that overexpression of IL-6 promotes resistance to enzalutamide through STAT3 activation, and autocrine IL-6 promotes AR transactivation through STAT3 induction. The authors also showed that STAT3 inhibition increases the sensitivity of LNCaP cells to enzalutamide.⁶² Therefore, STAT3 signaling appears to be another example of an AR-suppressed oncogenic pathway involved in the development of resistance to novel antiandrogens, and targeting STAT3 may be a reasonable treatment approach for patients with mCRPC.

Finally, Li et al found that the expression of c-Myb, a transcriptional factor upregulated in progression of various malignancies including prostate cancer,^{63,64} was increased with AR inhibition and antiandrogen therapy.⁶⁵ The authors also found that MYB silencing inhibited prostate cancer cell growth. Additionally, gene microarray data showed that c-Myb and AR share a subset of DNA damage response-related target genes, suggesting that c-Myb may replace AR as the dominant regulator of their common target genes in CRPC.⁶⁵ As such, c-Myb appears to regulate a resistance pathway that might be targeted to develop new treatment approaches for patients with mCRPC resistant to novel antiandrogens.

Overall, these results suggest that many alternative oncogenic signaling pathways likely play a role in the development



of a more aggressive form of CRPC. AR inhibition by novel antiandrogens may select for these cells, leading to rapid development of resistance and disease progression. However, exploiting these pathways in the clinic is still at a very preliminary stage, and the clinical relevance of these molecular insights remains uncertain at the present time.

Neuroendocrine/Small Cell Transdifferentiation

Neuroendocrine and small cell carcinoma of the prostate represent a subset of prostate cancer associated with aggressive tumor characteristics and particularly poor prognosis. Transformation of prostate adenocarcinoma to the neuroendocrine phenotype may be linked to resistance to AR signaling inhibition. Indeed, many cases of neuroendocrine prostate cancer, and even pure small cell prostate cancer, can arise after hormone therapy.⁶⁶

Recent studies have shown that the Aurora kinase A (*AURKA*) and N-myc (*MYCN*) gene abnormalities are associated with tumors at risk of progressing to neuroendocrine prostate cancer following hormonal therapy. In a study by Mosquera et al, *AURKA* amplification was identified in 65% of hormone-naïve and -treated prostate cancers that progressed to neuroendocrine carcinoma in 86% of metastases. Concurrent amplification of *MYCN* was present in about 70% of primary and treated prostate adenocarcinomas and 83% of metastases. In contrast, in an unselected prostate adenocarcinoma cohort, *AURKA* and *MYCN* amplifications were seen in only 5% of 169 cases.⁶⁷ Another study by Beltran et al showed significant overexpression and gene amplification of *AURKA* and *MYCN* in 40% of neuroendocrine prostate cancers compared with only 5% of prostate adenocarcinomas. These results suggest that *AURKA* and *MYCN* gene amplifications may cooperate to induce a neuroendocrine phenotype in prostate cells.⁶⁸ In addition, Svensson et al showed that treatment with enzalutamide led to a reduced expression of the repressor element-1 silencing transcription factor, a mediator of AR actions on gene repression that likely modulates neuroendocrine differentiation.⁶⁹

Several other molecular markers of small cell differentiation in prostate cancer have been identified. Tan et al showed loss of retinoblastoma protein in 90% of small cell carcinomas compared with only 7% of primary high-grade acinar carcinomas. Loss of PTEN protein was observed in 63% of small cell carcinomas, and *TP53* mutation was seen in 60% of cases.⁷⁰ The collaboration of retinoblastoma loss and p53 inactivation to drive neuroendocrine prostate cancers was also confirmed in a more recent study.⁷¹ Furthermore, preliminary results from the StandUp2Cancer initiative to molecularly characterize postabiraterone/enzalutamide CRPC has led to the recent discovery of a new histologic subtype of refractory prostate cancer. In this predominantly refractory patient population, there was a high preponderance of neuroendocrine (13%) histopathology, with an abundance of an additional nonadenocarcinoma

variant (27%) classified as *intermediate atypical carcinoma (IAC)*. This IAC pattern exhibits features that lie between those of neuroendocrine and small cell, with more neuronal genes, cell cycle genes, and less AR expression.⁷² The prognosis for patients harboring the IAC histology approximates that of small cell cancer and is much less favorable than an adenocarcinoma histology.

Programmed Death-Ligand 1/Programmed death-1 Upregulation

The importance of immune checkpoints in modulating antiandrogen resistance in prostate cancer is unclear. Recent studies have shown that treatment of certain prostate cancer cell lines with antiandrogens (including enzalutamide) can induce tumoral programmed death-ligand 1 (PD-L1) expression and that enzalutamide-resistant prostate cancer cell lines demonstrate striking expression of PD-L1.⁷³ These results are aligned with preclinical findings indicating increased amounts of circulating PD-L1/2-positive dendritic cells and increased levels of tumor-intrinsic PD-L1 in mice with enzalutamide-resistant tumors.^{73,74} As such, immune evasion of prostate cancer via upregulation of PD-L1 may possibly play a role in the development of resistance to novel antiandrogen therapy such as enzalutamide. To test this hypothesis, a phase 2 clinical trial is currently investigating the use of pembrolizumab (with continuation of enzalutamide) in men who develop secondary enzalutamide-refractory mCRPC (NCT02312557). Another trial will use the combination of ipilimumab and nivolumab in patients with AR-V7-positive mCRPC (NCT02601014).

Conclusion

mCRPC remains a lethal disease, despite the introduction of novel antiandrogen therapies that maximally decrease androgen ligand and AR signaling activity. While abiraterone and enzalutamide represent advances in the treatment of mCRPC and have demonstrated survival benefits, a significant proportion of patients have primary resistance to these agents and virtually all patients develop secondary resistance. This review has highlighted that various AR-dependent as well as AR-independent mechanisms likely play a role in the development of resistance to these novel antiandrogens, including upregulation of AR and CYP17, induction of AR splice variants, AR point mutations, upregulation of GR, activation of alternative oncogenic signaling pathways, neuroendocrine transformation, and immune evasion via PD-L1 upregulation. Targeting these pathways will be critical for the development of novel therapeutic approaches for patients with refractory mCRPC (Table 1) and will hopefully change the natural history of this disease. Developing robust clinical assays to simultaneously evaluate many of these resistance mechanisms at the same time (preferably from a single liquid biopsy)⁷⁵ is one of our major challenges for the future.

**Table 1.** Selected ongoing clinical trials attempting to overcome resistance to abiraterone and/or enzalutamide.

MECHANISM OF RESISTANCE	INVESTIGATIONAL AGENT(S)	TRIAL DESCRIPTION	TRIAL PHASE	NCT IDENTIFIER
AR/CYP17 upregulation	Abiraterone + Enzalutamide	Single-arm study evaluating the combination in mCRPC	Phase II	NCT01650194
	ARN-509 + Abiraterone	Non-randomized study of the combination in mCRPC	Phase I	NCT02123758
	ARN-509 + Everolimus	Open-label trial of the combination after progression on abiraterone	Phase I	NCT02106507
	ODM-201 (AR antagonist)	Double-blind, placebo-controlled trial in non-metastatic CRPC	Phase III	NCT02200614
	High-dose Testosterone [RESTORE]	Single-arm trial of high-dose testosterone for abiraterone/enzalutamide-refractory mCRPC	Phase II	NCT02090114
AR splice variants	EPI-506 (N-terminal domain inhibitor)	Single-arm trial in men with mCRPC after progression on enzalutamide or abiraterone	Phase I/II	NCT02606123
	Galeterone vs. Enzalutamide [ARMOR3]	Randomized trial of enzalutamide or galeterone in treatment-naïve, AR-V7-positive mCRPC	Phase III	NCT02438007
	Niclosamide + Enzalutamide	Open-label trial of the combination in AR-V7-positive mCRPC	Phase I	NCT02532114
Alternative oncogenic signaling pathways	Abiraterone + BEZ235 (PI3K inhibitor), or BKM120 (PI3K inhibitor)	Non-randomized study of abiraterone with either BEZ235 or BKM120 after progression on abiraterone	Phase Ib	NCT01634061
	Abiraterone + Cabozantinib (c-MET inhibitor)	Open-label trial of the combination in mCRPC	Phase I	NCT01574937
	Abiraterone + AT13387 (HSP90 inhibitor)	Randomized, open-label trial of AT13387 with/without abiraterone in men with mCRPC after progression on abiraterone	Phase I/II	NCT01685268
	Abiraterone + Dasatinib (Src inhibitor)	Randomized, open-label trial of abiraterone with/without dasatinib in mCRPC	Phase II	NCT01685125
	Abiraterone + Olaparib (PARP inhibitor)	Randomized, placebo-controlled trial of abiraterone with/without olaparib in mCRPC, chemotherapy resistant	Phase II	NCT01972217
	Abiraterone + GDC-0068 (Akt inhibitor) or GDC-0980 (PI3K/mTOR inhibitor)	Randomized, open-label trial of abiraterone with/without GDC-0068 or GDC-0980 in docetaxel-refractory mCRPC	Phase II	NCT01485861
	Abiraterone + Veliparib (PARP inhibitor)	Randomized, open-label trial of abiraterone with or without veliparib in mCRPC	Phase II	NCT01576172
	Abiraterone + AMG386 (angiopoietin inhibitor)	Randomized open-label trial of abiraterone with/without AMG386 in mCRPC	Phase II	NCT01553188
	Enzalutamide + GSK2636771 (PI3K inhibitor)	Single-arm trial of the combination in mCRPC patients progressing on enzalutamide w/PTEN loss	Phase I	NCT02215096
	Enzalutamide + Crizotinib (ALK and ROS-1 inhibitor)	Single-arm trial of the combination in mCRPC	Phase I	NCT02207504
	Enzalutamide + BI836845 (anti-IGF antibody)	Randomized, open-label trial of enzalutamide with/without BI836845 in mCRPC patients after abiraterone and docetaxel	Phase I/II	NCT02204072
	Enzalutamide + LY3023414 (dual PI3K/mTOR inhibitor)	Double-blinded, placebo-controlled trial of enzalutamide with/without LY3023414 in men progressing on abiraterone	Phase II	NCT02407054
	Neuroendocrine differentiation	Abiraterone + Alisertib (Aurora Kinase Inhibitor)	Open-label, single-arm trial of the combination after disease progression on abiraterone in mCRPC	Phase I/II
Immune evasion	Abiraterone + Ipilimumab (CTLA-4 inhibitor)	Single-arm trial of the combination in chemotherapy-naïve mCRPC	Phase II	NCT01688492
	Enzalutamide + ProstVac-VF (PSA-directed vaccine)	Randomized, open-label trial of enzalutamide with/without ProstVac-VF	Phase II	NCT01867333
	Pembrolizumab (anti PD-1)	Single-arm trial of pembrolizumab following progression on enzalutamide in mCRPC	Phase II	NCT02312557
	Ipilimumab (anti CTLA-4) + Nivolumab (anti PD-1) [STARVE]	Single-arm trial of combined immune checkpoint blockade in AR-V7-positive mCRPC	Phase II	NCT02601014



Author Contributions

Conceived and designed the experiments: KB, ESA. Analyzed the data: KB, ESA. Wrote the first draft of the manuscript: KB, ESA. Contributed to the writing of the manuscript: KB, ESA. Agree with manuscript results and conclusions: KB, ESA. Jointly developed the structure and arguments for the paper: KB, ESA. Made critical revisions and approved final version: KB, ESA. Both authors reviewed and approved of the final manuscript.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *CA Cancer J Clin*. 2015; 65(1):5–29.
2. Longo DL. New therapies for castration-resistant prostate cancer. *N Engl J Med*. 2010;363(5):479–81.
3. Ryan CJ, Smith MR, de Bono JS, et al; COU-AA-302 Investigators. Abiraterone in metastatic prostate cancer without previous chemotherapy. *N Engl J Med*. 2013;368(2):138–48.
4. O'Donnell A, Judson I, Dowsett M, et al. Hormonal impact of the 17 α -hydroxylase/C(17,20)-lyase inhibitor abiraterone acetate (CB7630) in patients with prostate cancer. *Br J Cancer*. 2004;90(12):2317–25.
5. Attard G, Reid AH, Yap TA, et al. Phase I clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. *J Clin Oncol*. 2008;26(28):4563–71.
6. Tran C, Ouk S, Clegg NJ, et al. Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science*. 2009;324(5928):787–90.
7. Scher HI, Beer TM, Higano CS, et al. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1–2 study. *Lancet*. 2010;375(9724):1437–46.
8. Scher HI, Fizazi K, Saad F, et al; AFFIRM Investigators. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med*. 2012;367(13):1187–97.
9. de Bono JS, Logothetis CJ, Molina A, et al; COU-AA-301 Investigators. Abiraterone and increased survival in metastatic prostate cancer. *N Engl J Med*. 2011;364(21):1995–2005.
10. Karantanos T, Evans CP, Tombal B, Thompson TC, Montironi R, Isaacs WB. Understanding the mechanisms of androgen deprivation resistance in prostate cancer at the molecular level. *Eur Urol*. 2015;67(3):470–9.
11. Maughan BL, Antonarakis ES. Androgen pathway resistance in prostate cancer and therapeutic implications. *Expert Opin Pharmacother*. 2015;16(10):1521–37.
12. Taplin ME, Bublej GJ, Shuster TD, et al. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. *N Engl J Med*. 1995; 332(21):1393–8.
13. Mostaghel EA, Marck BT, Plymate SR, et al. Resistance to CYP17A1 inhibition with abiraterone in castration-resistant prostate cancer: induction of steroidogenesis and androgen receptor splice variants. *Clin Cancer Res*. 2011;17(18):5913–25.
14. Yamamoto Y, Lloriot Y, Beraldi E, et al. Generation 2.5 antisense oligonucleotides targeting the androgen receptor and its splice variants suppress enzalutamide-resistant prostate cancer cell growth. *Clin Cancer Res*. 2015;21(7):1675–87.
15. Azad AA, Volik SV, Wyatt AW, et al. Androgen receptor gene aberrations in circulating cell-free DNA: biomarkers of therapeutic resistance in castration-resistant prostate cancer. *Clin Cancer Res*. 2015;21(10):2315–24.
16. Romanel A, Tandefelt DG, Conteduca V, et al. Plasma AR and abiraterone-resistant prostate cancer. *Sci Transl Med*. 2015;7(312):312re10.
17. Locke JA, Guns ES, Lubik AA, et al. Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. *Cancer Res*. 2008;68(15):6407–15.
18. Salvi S, Casadio V, Conteduca V, et al. Circulating cell-free AR and CYP17A1 copy number variations may associate with outcome of metastatic castration-resistant prostate cancer patients treated with abiraterone. *Br J Cancer*. 2015;112(10): 1717–24.
19. Sharifi N, McPhaul MJ, Auchus RJ. “Getting from here to there” – mechanisms and limitations to the activation of the androgen receptor in castration-resistant prostate cancer. *J Invest Med*. 2010;58(8):938–44.
20. Dehm SM, Schmidt LJ, Heemers HV, Vessella RL, Tindall DJ. Splicing of a novel androgen receptor exon generates a constitutively active androgen receptor that mediates prostate cancer therapy resistance. *Cancer Res*. 2008;68(13):5469–77.
21. Nadiminty N, Tummala R, Liu C, et al. NF- κ B2/p52 induces resistance to enzalutamide in prostate cancer: role of androgen receptor and its variants. *Mol Cancer Ther*. 2013;12(8):1629–37.
22. Lu C, Luo J. Decoding the androgen receptor splice variants. *Transl Androl Urol*. 2013;2(3):178–86.
23. Robinson D, Van Allen EM, Wu YM, et al. Integrative clinical genomics of advanced prostate cancer. *Cell*. 2015;161(5):1215–28.
24. Li Y, Hwang TH, Oseth LA, et al. AR intragenic deletions linked to androgen receptor splice variant expression and activity in models of prostate cancer progression. *Oncogene*. 2012;31(45):4759–67.
25. Hu R, Dunn TA, Wei S, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormone-refractory prostate cancer. *Cancer Res*. 2009;69(1):16–22.
26. Hu R, Lu C, Mostaghel EA, et al. Distinct transcriptional programs mediated by the ligand-dependent full-length androgen receptor and its splice variants in castration-resistant prostate cancer. *Cancer Res*. 2012;72(14):3457–62.
27. Antonarakis ES, Lu C, Wang H, et al. AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. *N Engl J Med*. 2014;371(11):1028–38.
28. Steinestel J, Luedeke M, Arndt A, et al. Detecting predictive androgen receptor modifications in circulating prostate cancer cells. *Oncotarget*. 2015. [Epub ahead of print]. Doi: 10.18632/oncotarget.3925
29. Efstathiou E, Titus M, Wen S, et al. Molecular characterization of enzalutamide-treated bone metastatic castration-resistant prostate cancer. *Eur Urol*. 2015;67(1): 53–60.
30. Barbieri CE, Baca SC, Lawrence MS, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nat Genet*. 2012;44(6): 685–9.
31. Taplin ME, Rajeshkumar B, Halabi S, et al; Cancer and Leukemia Group B Study 9663. Androgen receptor mutations in androgen-independent prostate cancer: cancer and leukemia group B study 9663. *J Clin Oncol*. 2003;21(14):2673–8.
32. Taylor BS, Schultz N, Hieronymus H, et al. Integrative genomic profiling of human prostate cancer. *Cancer Cell*. 2010;18(1):11–22.
33. Grasso CS, Wu YM, Robinson DR, et al. The mutational landscape of lethal castration-resistant prostate cancer. *Nature*. 2012;487(7406):239–43.
34. Hay CW, McEwan IJ. The impact of point mutations in the human androgen receptor: classification of mutations on the basis of transcriptional activity. *PLoS One*. 2012;7(3):e32514.
35. Gottlieb B, Beitel LK, Nadarajah A, Paliouras M, Trifiro M. The androgen receptor gene mutations database: 2012 update. *Hum Mutat*. 2012;33(5):887–94.
36. Taplin ME, Bublej GJ, Ko YJ, et al. Selection for androgen receptor mutations in prostate cancers treated with androgen antagonist. *Cancer Res*. 1999;59(11):2511–5.
37. Steketee K, Timmerman L, Ziel-van der Made AC, Doesburg P, Brinkmann AO, Trapman J. Broadened ligand responsiveness of androgen receptor mutants obtained by random amino acid substitution of H874 and mutation hot spot T877 in prostate cancer. *Int J Cancer*. 2002;100(3):309–17.
38. Culig Z, Hobisch A, Cronauer MV, et al. Mutant androgen receptor detected in an advanced-stage prostatic carcinoma is activated by adrenal androgens and progesterone. *Mol Endocrinol*. 1993;7(12):1541–50.
39. Culig Z, Hoffmann J, Erdel M, et al. Switch from antagonist to agonist of the androgen receptor bicalutamide is associated with prostate tumour progression in a new model system. *Br J Cancer*. 1999;81(2):242–51.
40. Chen EJ, Sowalsky AG, Gao S, et al. Abiraterone treatment in castration-resistant prostate cancer selects for progesterone responsive mutant androgen receptors. *Clin Cancer Res*. 2015;21(6):1273–80.
41. Joyce R, Fenton MA, Rode P, et al. High dose bicalutamide for androgen independent prostate cancer: effect of prior hormonal therapy. *J Urol*. 1998;159(1): 149–53.
42. Balbas MD, Evans MJ, Hosfield DJ, et al. Overcoming mutation-based resistance to antiandrogens with rational drug design. *Elife*. 2013;2:e00499.
43. Korpai M, Korn JM, Gao X, et al. An F876 L mutation in androgen receptor confers genetic and phenotypic resistance to MDV3100 (enzalutamide). *Cancer Discov*. 2013;3(9):1030–43.
44. Joseph JD, Lu N, Qian J, et al. A clinically relevant androgen receptor mutation confers resistance to second-generation antiandrogens enzalutamide and ARN-509. *Cancer Discov*. 2013;3(9):1020–9.
45. Arora VK, Schenkein E, Murali R, et al. Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell*. 2013;155(6): 1309–22.
46. Richards J, Lim AC, Hay CW, et al. Interactions of abiraterone, eplerenone, and prednisolone with wild-type and mutant androgen receptor: a rationale for increasing abiraterone exposure or combining with MDV3100. *Cancer Res*. 2012;72(9):2176–82.
47. Carreira S, Romanel A, Goodall J, et al. Tumor clone dynamics in lethal prostate cancer. *Sci Transl Med*. 2014;6(254):254ra125.
48. Sahu B, Laakso M, Pihlajamaa P, et al. FoxA1 specifies unique androgen and glucocorticoid receptor binding events in prostate cancer cells. *Cancer Res*. 2013;73(5): 1570–80.
49. Davies P, Rushmere NK. Association of glucocorticoid receptors with prostate nuclear sites for androgen receptors and with androgen response elements. *J Mol Endocrinol*. 1990;5(2):117–27.
50. Ueda T, Mawji NR, Bruchovsky N, Sadar MD. Ligand-independent activation of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells. *J Biol Chem*. 2002;277(41):38087–94.



51. Feng S, Tang Q, Sun M, Chun JY, Evans CP, Gao AC. Interleukin-6 increases prostate cancer cells resistance to bicalutamide via TIF2. *Mol Cancer Ther.* 2009; 8(3):665–71.
52. Culig Z, Hobisch A, Cronauer MV, et al. Androgen receptor activation in prostatic tumor cell lines by insulin-like growth factor-I, keratinocyte growth factor, and epidermal growth factor. *Cancer Res.* 1994;54(20):5474–8.
53. Mellingerhoff IK, Vivanco I, Kwon A, Tran C, Wongvipat J, Sawyers CL. HER2/neu kinase-dependent modulation of androgen receptor function through effects on DNA binding and stability. *Cancer Cell.* 2004;6(5):517–27.
54. Chen L, Mooso BA, Jathal MK, et al. Dual EGFR/HER2 inhibition sensitizes prostate cancer cells to androgen withdrawal by suppressing ErbB3. *Clin Cancer Res.* 2011;17(19):6218–28.
55. Carver BS, Chapinski C, Wongvipat J, et al. Reciprocal feedback regulation of PI3K and androgen receptor signaling in PTEN-deficient prostate cancer. *Cancer Cell.* 2011;19(5):575–86.
56. Zhang W, Zhu J, Efferon CL, et al. Inhibition of tumor growth progression by antiandrogens and mTOR inhibitor in a pten-deficient mouse model of prostate cancer. *Cancer Res.* 2009;69(18):7466–72.
57. Kinkade CW, Castillo-Martin M, Puzio-Kuter A, et al. Targeting AKT/mTOR and ERK MAPK signaling inhibits hormone-refractory prostate cancer in a pre-clinical mouse model. *J Clin Invest.* 2008;118(9):3051–64.
58. Zhong J, Ding L, Bohrer LR, et al. p300 acetyltransferase regulates androgen receptor degradation and PTEN-deficient prostate tumorigenesis. *Cancer Res.* 2014;74(6):1870–80.
59. Debes JD, Schmidt LJ, Huang H, Tindall DJ. P300 mediates androgen-independent transactivation of the androgen receptor by interleukin 6. *Cancer Res.* 2002;62(20):5632–6.
60. Abdulghani J, Gu L, Dagvadorj A, et al. Stat3 promotes metastatic progression of prostate cancer. *Am J Pathol.* 2008;172(6):1717–28.
61. Schroeder A, Herrmann A, Cherryholmes G, et al. Loss of androgen receptor expression promotes a stem-like cell phenotype in prostate cancer through STAT3 signaling. *Cancer Res.* 2014;74(4):1227–37.
62. Liu C, Zhu Y, Lou W, Cui Y, Evans CP, Gao AC. Inhibition of constitutively active Stat3 reverses enzalutamide resistance in LNCaP derivative prostate cancer cells. *Prostate.* 2014;74(2):201–9.
63. Edwards J, Krishna NS, Grigor KM, Bartlett JM. Androgen receptor gene amplification and protein expression in hormone refractory prostate cancer. *Br J Cancer.* 2003;89(3):552–6.
64. Stenman G, Andersson MK, Andren Y. New tricks from an old oncogene: gene fusion and copy number alterations of MYB in human cancer. *Cell Cycle.* 2010;9(15):2986–95.
65. Li L, Chang W, Yang G, et al. Targeting poly(ADP-ribose) polymerase and the c-myc-regulated DNA damage response pathway in castration-resistant prostate cancer. *Sci Signal.* 2014;7(326):ra47.
66. Aparicio A, Logothetis CJ, Maity SN. Understanding the lethal variant of prostate cancer: power of examining extremes. *Cancer Discov.* 2011;1(6):466–8.
67. Mosquera JM, Beltran H, Park K, et al. Concurrent AURKA and MYCN gene amplifications are harbingers of lethal treatment-related neuroendocrine prostate cancer. *Neoplasia.* 2013;15(1):1–10.
68. Beltran H, Rickman DS, Park K, et al. Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. *Cancer Discov.* 2011; 1(6):487–95.
69. Svensson C, Ceder J, Iglesias-Gato D, et al. REST mediates androgen receptor actions on gene repression and predicts early recurrence of prostate cancer. *Nucleic Acids Res.* 2014;42(2):999–1015.
70. Tan HL, Sood A, Rahimi HA, et al. Rb loss is characteristic of prostatic small cell neuroendocrine carcinoma. *Clin Cancer Res.* 2014;20(4):890–903.
71. Aparicio AM, Shen L, Li Ning Tapia EM, et al. Combined tumor suppressor defects characterize clinically defined aggressive variant prostate cancers. *Clin Cancer Res.* 2015. [Epub ahead of print]. Doi: 10.1158/1078-0432.CCR-15-1259
72. Aggarwal RR, Thomas G, Youngren J, et al. Androgen receptor (AR) amplification in patients (pts) with metastatic castration resistant prostate cancer (mCRPC) resistant to abiraterone (abi) and enzalutamide (enz): preliminary results from the SU2C/PCF/AACR West Coast Prostate Cancer Dream Team (WCDT). *J Clin Oncol.* 2015;33(suppl):abstr5068.
73. Bishop JL, Sio A, Angeles A, et al. PD-L1 is highly expressed in enzalutamide resistant prostate cancer. *Oncotarget.* 2015;6(1):234–42.
74. Ramos RN, de Moraes CJ, Zelante B, Barbuto JA. What are the molecules involved in regulatory T-cells induction by dendritic cells in cancer? *Clin Dev Immunol.* 2013;2013:806025.
75. Schweizer MT, Antonarakis ES. Liquid biopsy: clues on prostate cancer drug resistance. *Sci Transl Med.* 2015;7(312):312fs45.