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2015 Dec; 41 (10): 884-92 doi: 10.1016/j.ctrv.2015.08.002

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Highlights

- Enzalutamide and abiraterone prolong OS, but resistance is a major clinical problem.
- Mechanisms of *de novo* or acquired resistance can be AR-dependent or AR-independent.
- To prevent or overcome resistance, many new agents are currently being tested.
- The addition of these drugs may improve synergically the efficacy of hormonal therapy.

Abstract

In recent years, in castration resistant prostate cancer (CRPC), several new drugs have been approved that prolong overall survival, including enzalutamide and abiraterone, two new-generation hormonal therapies.

Despite the demonstrated benefit of these agents, not all patients with CRPC are responsive to treatment, the gain in median progression-free survival with these therapies compared to standard of care is, rather disappointingly, still less than six months and the appearance of acquired resistance is almost universal. Approximately one third of patients treated with abiraterone and 25% of those treated with enzalutamide show primary resistance to these agents. Even if the mechanisms of resistance to these agents are not fully defined, many hypotheses are emerging, including systemic and intratumoral androgen biosynthesis up-regulation, androgen receptor (AR) gene mutations and amplifications, alteration of pathways involved in cross-talk with AR signaling, glucocorticoid receptor overexpression, neuroendocrine differentiation, immune system deregulation and others.

The aim of this paper is to review currently available data about mechanisms of resistance to abiraterone and enzalutamide, and to discuss how these mechanisms could be potentially overcome through novel therapeutic agents.

Keywords

- Prostate cancer;
- Abiraterone;
- Enzalutamide;
- Mechanisms of resistance

Introduction

With nearly 220,000 new cases expected in the USA in 2015, prostate cancer (PC) represents the most frequent type of cancer in men, accounting for almost 26% of new cancer cases [1]. Despite most of the new patients are diagnosed in early stage, approximately 4% of the cases at baseline are in advanced stage and some patients treated initially with radical surgery will subsequently relapse.

Since the seminal work of Huggins and Hodges [2], androgen deprivation therapy (ADT), either surgical or biochemical, has been the mainstay of treatment for relapsed and metastatic PC patients, due to the driving role of androgen receptor (AR) in the development of these tumors.

Despite the initial high response rates, many patients become castrate-resistant within 3 years from the start of ADT and, consequently, there is a growing interest in testing new agents and strategies to overcome acquired resistance.

Recently it has been shown that, in castration resistant PC (CRPC) patients, even small concentrations of extra-gonadal androgens persisting despite ADT could activate the AR pathway and that PC cells could

synthesize their own androgens [3]. Following these observations, a renewed interest on the AR signaling pathway was generated, and a second generation of hormonal therapies has been developed.

Abiraterone acetate is an oral drug that selectively and irreversibly inhibits the CYP17A1 microsomal enzyme, leading to the inhibition of testosterone biosynthesis in adrenal glands, in testis and in PC cells. Enzalutamide is an oral AR antagonist with an 8-fold higher affinity for its target than bicalutamide, preventing also AR nuclear translocation and DNA binding.

In CRPC, patients pretreated with docetaxel, two phase III trials, COU-AA-301 and AFFIRM, showed an improvement in overall survival (OS) with abiraterone (median OS 14.8 vs 10.9 months; hazard ratio [HR] 0.65, 95% CI, 0.54–0.77; $p < 0.001$) [4] and enzalutamide (median OS 18.4 vs 13.6 months; HR 0.63, 95% CI, 0.53–0.75; $p < 0.001$) [5], respectively. More recently, the COU-AA-302 study confirmed the efficacy of abiraterone in asymptomatic and mildly symptomatic chemotherapy-naive metastatic CRPC patients in terms of OS (median OS 34.7 vs 30.3 months; HR: 0.81, 95% CI, 0.70–0.93; $p = 0.0033$) [6].

In the PREVAIL study, enzalutamide showed a statistically significant 29% reduction in the risk of death (HR 0.70, 95% CI, 0.59–0.83; $p = 0.0001$) and an 81% reduction in the risk of radiographic progression compared to placebo (HR 0.19; CI 95%, 0.15–0.23; $p < 0.0001$) [7].

Based on these data, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) granted approval for abiraterone for the treatment of both docetaxel-naive and docetaxel-pretreated CRPC patients and for enzalutamide in the post-docetaxel setting.

Despite the inhibitory properties of these agents, PC cells, even after initial response, become resistant and cause clinical disease progression, posing the question how to deal with acquired resistance. In this paper we will review the putative mechanisms of primary and acquired resistance to new hormonal agents and some potential new strategies to overcome it.

Physiological androgen synthesis

In adult men, testosterone (T) and 5-α-dihydrotestosterone (DHT), mainly produced in the testes, with only 5–10% being synthesized from the adrenal glands, are the commonest forms of endogenous androgens and the major physiological AR ligands. Other steroid hormones, also produced by the adrenal glands, such as androstenedione and dehydroepiandrosterone (DHEA), can bind the AR.

In peripheral tissues, weak adrenal androgens such as DHEA-sulfate (DHEA-S) can be reduced to T, even if this biochemical transformation seems to be a negligible source of T in men with intact testes.

In the adrenal glands and testes, all steroid hormones, including T, are synthesized from cholesterol as initial 27-carbon substrate, through sequential reactions catalyzed by several enzymes.

Pregnenolone is generated from cholesterol and the biochemical reaction is catalyzed by the cholesterol side chain cleavage mitochondrial enzyme CYP11A1. The Leydig cells of the testis, the theca and granulosa cells of the ovary, the adrenal cortex cells have sufficient CYP11A1 levels to significantly contribute to the whole pool of circulating steroids.

The enzyme 3β-hydroxysteroid dehydrogenase 1 (HSD3B1), expressed in peripheral tissues such as prostate basal epithelium and the enzyme 3β-hydroxysteroid dehydrogenase 2 (HSD3B2), expressed in steroidogenic organs, are two iso-enzymes responsible for the oxidation of 3β-hydroxy-D^5-steroids to their 3-keto-D^4-congeners. In fact these iso-enzymes isomerize the double bond between C5 and C6 to that between C4 and C5, resulting in the specific conversion from pregnenolone to progesterone, from 17-OH-pregnenolone to 17-OH-progesterone and from DHEA to androstenedione (AD).

Another enzyme acting on pregnenolone is the 17α-hydroxylase/17,20-lyase (CYP17A1), a microsomal enzyme with two functional activities: 17alpha-hydroxylase which catalyzes the 17alpha-hydroxylation of C21 steroids and 17,20-lyase which catalyzes C17–21 bond scission, converting C21 compounds to C19 steroids both in testis and adrenal glands.

The sequential reactions catalyzed by CYP17A1 result in the synthesis of oxidized (17-keto) androgens such as DHEA from pregnenolone and AD from progesterone. In the adrenal glands AD and DHEA are the major substrates for aldo–keto reductase 1 (AKR1C3), enzyme responsible for the reduction of AD to T and the reduction of DHEA to androstenediol, then converted to T by HSD3B2.A different enzyme, the 17-β-hydroxy-steroid dehydrogenase type 3 (HSD17B3), mediates T synthesis from DHEA in the Leydig cells of the testes.

The final reaction of the sex steroid synthetic pathway is the 5 α reduction of circulating T to the more potent androgen 5 α-dihydrotestosterone (DHT) in genital tissues and prostate, catalyzed by the type 1 and type 2 isoforms of steroid 5α-reductase (SRD5A1, SRD5A2). This represents the classical pathway of androgen biosynthesis.

DHT synthesis can be obtained, without intermediate T synthesis, through two other pathways: the first one called “backdoor pathway”, that consists in eight enzymatic steps and synthesizes androsterone from progesterone or 17-OH-progesterone and then synthesizes DHT via 3α-androstanediol production. The second is the alternative pathway that leads to DHT synthesis from 5-alpha androstenedione through the enzyme AKR1C3 (Fig. 1) [8].

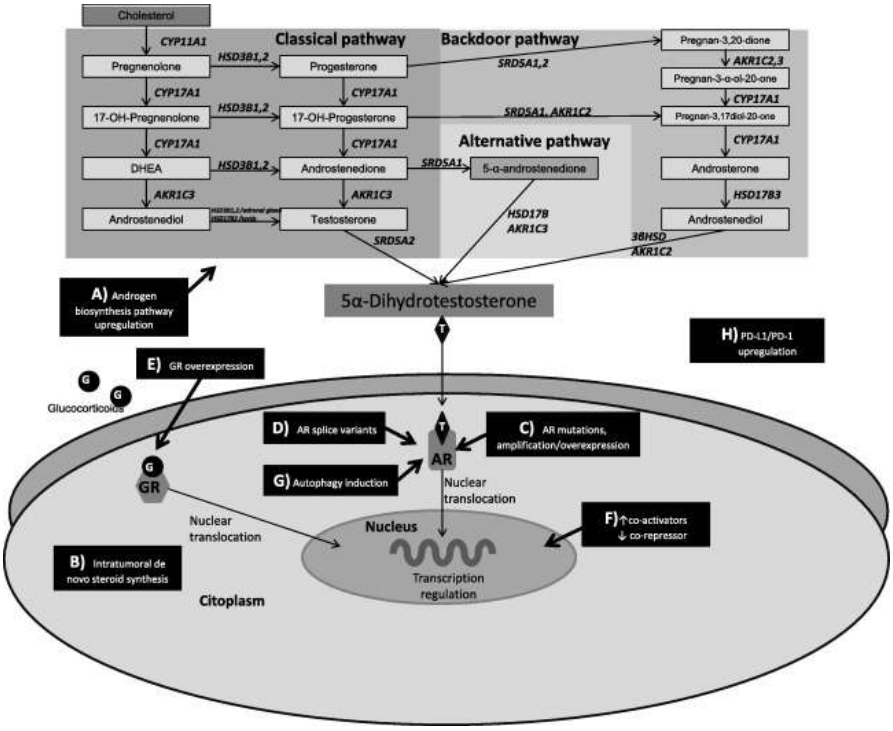

Fig. 1.

Physiological androgen synthesis and suggested mechanisms of resistance to enzalutamide and abiraterone. (A) Androgen biosynthesis pathways upregulazion, (B) intratumoral de novo steroid synthesis, (C) AR mutations, amplification and overexpression, (D) AR slice variants, (E) GR overexpression, (F) Co-activators increase and co-repressor decrease, (G) autophagy induction, (H) PD-L1/PD-1 upregulation. AR: androgen receptor, T: dihydrotestosterone, G: glucocorticoids, GR: glucocorticoids receptor

Figure options

The androgen receptor

The AR gene is located on chromosome Xq11–12 and accounts for eight exons. AR is a 110-kDa nuclear protein that contains 918 acid residues and binds a specific hormone response element (HRE) known as androgen response element (ARE). AR is formed by different domains: the N-terminal domain (NTD), the DNA-binding domain (DBD), the hinge region and the ligand-binding domain (LBD) [9].

Once activated, AR binds AREs as a dimer. There are two different AREs: the inverted repeat AREs, that bind and mediate transcriptional activation of both glucocorticoid receptor (GR) and AR, and the direct repeat AREs that are specific for AR. Nuclear translocation and DNA-binding properties of AR are also enhanced by phosphorylation on NTD [10] and by the interaction with chaperones [11].

AR binding with testosterone and DHT results in a conformational change of AR leading to a tight homodimeric binding to AREs. Many co-activators and co-repressors modulates this interaction and, at the same time, allow a cross-talk with several other pathways involved in cellular signaling and apoptosis [12].

The adaptive response to androgen deprivation

Recently, a significant shift in the understanding of natural history of PC has occurred and it is currently known that castration resistance does not necessarily results in resistance to hormonal agents. In fact, despite the dramatic decrease of serum T induced by ADT, CRPC remains driven by AR signaling, being able to overexpress androgen dependent genes [13].

Multiple mechanisms have been identified to explain this phenomenon, including AR gene mutation [14], AR gene overexpression [15], AR splice variants expression [16], increased expression of transcriptional co-activators [14].

Intraprostatic synthesis can be a significant source of androgen under the selective pressure of endocrine therapy [17]. Several studies showed that intraprostatic androgen levels remain significantly elevated despite ADT [18], [19], [20] and [21]. One of these study demonstrated that patients had a 95% reduction in T serum levels corresponding only to a 70–80% reduction in intraprostatic T and DHT [21]. Data in metastatic CRPC tumor samples showed that tumor progression was associated to the up-regulation of steroidogenic enzymes [22] and [23] and intratumoral androgens accumulation [24].

PC cells can synthesize androgens through two mechanisms: the first through conversion from androgen precursors derived from adrenal glands such as androstenedione, DHEA and DHEA-S [22] and the second way is represented from the *de novo* synthesis from cholesterol [23].

The mechanisms of resistance to abiraterone or enzalutamide

Despite the demonstrated benefits of both abiraterone and enzalutamide, some CRPC patients are primary resistant and do not respond to treatment, the gain in progression-free survival and OS obtained with these therapies is clinically relevant but limited, and the appearance of acquired resistance is the rule for all treated patients.

Although primary resistance has been defined by some authors as the absence of PSA reduction [25], a commonly accepted definition of primary resistance is a treatment failure within the first months (usually 3) after initiation, as a result of overt clinical progression, with or without imaging progression [26]. According to this conventional definition of primary resistance, treatment failures that occur later are considered acquired resistance.

Since a significant proportion of patients treated with enzalutamide and abiraterone showed primary resistance, the understanding of the biology of these mechanisms is crucial in order to develop new treatment strategies. The percentage of patients with primary resistance in published phase III studies with CYP17A1 inhibitors (abiraterone [4] and [6] and orteronel [27] and [28]) and AR inhibitors (enzalutamide [5] and [7]), defined as the probability of radiographic progression within the first 3 months, are reported in Table 1. Several mechanisms of acquired and primary resistance have been reported, including systemic and intratumoral androgen biosynthesis up-regulation, AR gene mutations and amplifications, alteration of pathways involved in cross-talk with AR signaling, glucocorticoid receptor overexpression, neuroendocrine differentiation and immune system deregulation (Table 2).

Table 1.

Percentage of patients with primary resistance (defined as probability of radiographic progression within the first 3 months) in published phase III studies with abiraterone, enzalutamide and orteronel.

Study	Probability of early radiographic progression (within the first 3 months)[a]	
	Experimental arm	**Control arm**
Chemotherapy-naïve		
Abiraterone plus prednisone[6]	Less than 10% of patients	(Placebo plus prednisone): about 20% of patients
Enzalutamide [7]	Less than 10% of patients	(Placebo): about 30% of patients
Orteronel plus prednisone [28]	Less than 10% of patients	(Placebo plus prednisone): slightly more than 10% of patients
Pretreated with chemotherapy		
Abiraterone plus prednisone [4]	Slightly less than 40% of patients	(Placebo plus prednisone): slightly less than 50% of patients
Enzalutamide [5]	Slightly more than 20% of patients	(Placebo): about 50% of patients
Orteronel plus prednisone [27]	About 15% of patients	(Placebo plus prednisone): about 20% of patients

Probability of radiographic progression within the first 3 months is estimated from Kaplan–Meier curves of radiographic progression-free survival.

Table options

Table 2.

Proposed mechanisms of resistance to abiraterone and enzalutamide.

Abiraterone	Enzalutamide
AR amplification/overexpression	AR amplification/overexpression
AR mutations	AR mutations
AR activation by exogenous corticosteroids and steroid precursors upstream CYP17A1	AR splice variants
AR splice variants	Glucocorticoid receptor overexpression
Androgen biosynthesis pathway upregulation	Intracrine synthesis of androgens
Glucocorticoid receptor overexpression	Crosstalk with growth factor
Intracrine synthesis of androgen	Neuroendocrine transformation
Neuroendocrine transformation	Autophagy induction
Autophagy induction	Immune evasion
Immune evasion	

Table options

Androgen biosynthesis pathway up-regulation and intratumor *de novo* steroid synthesis

Studies with CRPC xenografts have shown that several genes involved in the androgen synthesis pathway are upregulated during treatment with abiraterone such as a 2-fold overexpression of CYP17A1 gene [29].

Studies in animal models have also shown that tumor relapse on abiraterone was associated with further upregulation of intratumor CYP17A1 and other key genes involved in intratumor androgen synthesis, such as CYP11A1, AKR1C3 and HSD17B3 [30].

Abiraterone does not completely ablate serum precursor steroids such as DHEA-S, which in PC cells could be transported and metabolized to DHEA by steroid sulfatase. Therefore, tumor cells exposed to abiraterone increase their ability to carry in and metabolize steroid precursors downstream of CYP17A1.

A gain-of-function mutation (N367T) was shown to produce 3 beta-hydroxysteroid dehydrogenase type 1 resistant to ubiquitination, which results in the accumulation of this enzyme responsible for DHT synthesis during treatment with abiraterone [31]. Moreover, DHT can be synthesized from 5-alpha-androstenedione instead of T through the activity of the enzyme AKR1C3 that is over-expressed in CRPC [15].

It has been reported that AR activity in the castration resistant VcaP xenografts is driven by CYP17A1 and AKR1C3-dependent *de novo* intratumor androgen synthesis from cholesterol and in tumor biopsies from CRPC patients treated with CYP17A1 inhibitor therapy intratumor expression of CYP17A1 was increased. CRPC cells expressing a progesterone responsive T878A mutant AR are not CYP17A1-dependent, but AR activity in these cells is steroid dependent yet and mediated by upstream CYP11A1-dependent intratumor pregnenolone/progesterone synthesis [17].

AKR1C3 is overexpressed also in enzalutamide resistant PC cells and this overexpression confers resistance to enzalutamide, while down-regulation of AKR1C3 in PC cells induces sensitization to the same agent. Moreover indomethacin, a potent AKR1C3 inhibitor, re-sensitizes enzalutamide resistant PC cells to enzalutamide both *in vivo* and *in vitro* [32].

Androgen receptor mutations

As a consequence of the androgen deprivation and androgen receptor antagonism, clonal selection of tumor cells can determine the expansion of both AR somatic mutation and aberrant transcription. The frequency of AR mutations, which is about 8–25% in CRPC treated with ADT, may become a even more relevant resistance mechanism in the context of more effective suppression of AR signaling.

Mutations in the AR ligand-binding domain can alter its structure and enable its activation by alternative ligands including progesterone, hydrocortisone, estradiol and certain AR antagonists [33].

Since serum levels of progesterone and other upstream steroids are increased during treatment with CYP17A1 inhibitors mutant AR, that are activated by these upstream steroids, may represent a relevant resistance mechanism. A specific AR mutation, L701H (or L701 coupled with T878A) warrants the AR to respond to glucocorticoids and progesterone [34] and [35] and CRPC cells expressing this progesterone and glucocorticoid responsive T878A mutant AR are resistant to CYP17A1 inhibition [17] and [35].

The results of a phase I/II study of abiraterone in chemotherapy-naïve patients support the hypothesis of AR activation by other steroids [32]. In that trial, dexamethasone was added at the time of disease progression and led to the reversion of resistance in 33% of patients regardless of prior treatment with dexamethasone, quite likely related to a reduction of ACTH-mediated stimulation of steroid synthesis. However, it should be also taken into account that the exogenous glucocorticoid or mineralocorticoid antagonists administered to control abiraterone side effects may contribute to activate mutant AR [34].

Studies in PC cell lines treated with enzalutamide and ARN-509 have identified a new missense mutation (F876L) in the LBD of the AR, that confers an antagonist-to-agonist switch driving the resistance to enzalutamide and ARN-509 while maintains sensitivity to first generation agents such as bicalutamide [36], [37] and [38].

Androgen receptor amplification/overexpression

More than 80% of CRPC show high levels of AR expression because of gene amplification and/or overexpression [33] Preclinical studies showed that treatment with abiraterone is associated with a 3-fold increased expression of both full-length AR (FL AR) and truncated AR variants [29].

Enzalutamide resistant LNCaP cells express high levels of both FL AR and AR variants compared to CRPC LNCaP and knockdown of FL AR alone, or FL AR plus ARVs, induces apoptosis, suppresses cell growth and AR-regulated gene expression and delays tumor growth *in vivo*, suggesting that the AR is the key driver in the enzalutamide-resistant LNCaP model [39].

Androgen receptor splice variants (ARV)

In several preclinical models, activation of the AR in the absence of ligand occurs with AR splice variants that lack the LBD remaining constitutively active as a transcription factor in a ligand-independent manner [40].

The most commonly expressed variants that drive PC progression under ADT conditions are ARV-7 and ARV-567 [41]. It is known that ARV levels are correlated with PC progression and that ARV expression significantly increases during ADT [16], [42], [43], [44] and [45].

In CRPC xenografts, both abiraterone and enzalutamide are associated with increased expression of truncated variants [29] and [39] and ARV expression can mediate resistance to therapies targeting FL AR, including enzalutamide. Metastatic CRPC cell lines expressing ARV show increased growth when exposed to enzalutamide and the selective knockdown of ARV expression inhibits androgen independent growth and restores responsiveness to androgens and antiandrogens [46].

Antonarakis et al. have recently isolated ARV-7 in circulating tumor cells from 31 enzalutamide-treated and 31 abiraterone-treated CRPC patients. ARV-7-positive patients had lower PSA response rates than ARV-7-negative both in patients treated with enzalutamide (0% vs 53%, $p = 0.004$) and in those treated with abiraterone (0% vs 68%, $p = 0.004$). Among patients receiving enzalutamide median clinical or radiographic PFS in ARV-7 positive group was 2.1 months compared to 6.1 months in the ARV-7 negative group (HR: 8.5; 95% CI: 2.8–25.5; $p < 0.001$). Similar results were obtained in patients receiving abiraterone (median PFS: 2.3 vs 6.3 months; HR 16.5; 95% CI: 3.3–82.9; $p < 0.001$). Moreover, ARV-7 was present in 9–15% of treatment-naive mCRPC patients but it increased during treatment with abiraterone (55%) or enzalutamide (50%), supporting the hypothesis that both intrinsic and acquired resistance to these agents may be associated with ARV-7 [47] and [48].

In a recent study Liu et al. identified niclosamide, an FDA-approved anthelminthic drug, as a potent ARV-7 inhibitor in PC cells. Niclosamide significantly inhibited ARV-7 protein expression by protein degradation through a proteasome-dependent pathway and ARV-7 transcription activity and reduced ARV-7 recruitment to the PSA promoter. Niclosamide inhibits *in vitro* PC cell growth and induces cell apoptosis and its combination with enzalutamide resulted in a significant inhibition of enzalutamide-resistant tumor growth, suggesting that niclosamide enhances enzalutamide activity and overcomes enzalutamide resistance in CRPC cells [49].

Glucocorticoid receptor overexpression

Acquired resistance to enzalutamide and abiraterone can occur through an increased expression of glucocorticoid receptor (GR) that shares response elements with AR in multiple gene targets.

Arora et al. identified that GR overexpression confers clinical resistance to enzalutamide [50]. GR is able to drive expression of certain AR target genes independent of AR and the GR agonist dexamethasone induced *in vitro* enzalutamide resistance whereas a GR antagonist restored sensitivity [50]. However, the prevalence and the clinical relevance of GR-driven CRPC are unknown.

The progesterone receptor (PR) has been shown to be increased in CRPC and its two isoforms A and B are expressed in stromal fibroblast and smooth muscle cells of the prostate and are known to regulate cell proliferation. PR may be involved in resistance to enzalutamide and abiraterone due to continue progesterone production [51].

Activation of other pathways

Parallel pathways have been also implicated in acquired resistance to abiraterone and enzalutamide, including EGFR-, Scr- and Pi3K pathway [52], [53] and [54], that may crosstalk with the AR pathway.

Activation of Pi3K-AKT-mTOR signaling pathway is strongly implicated in prostate cancer progression [55] and [56] and this pathway contributes to PC development and progression though interaction with other critical pathways. In preclinical models Carver et al. demonstrated that AR and Pi3K signaling pathways are cross-regulated by reciprocal feedback. AR pathway inhibition results in a stronger activation of the Pi3K pathway, and may promote resistance to AR pathway inhibitors. Moreover Thomas et al. showed *in vivo* that synergistic targeting of Pi3K/AKT pathway and AR axis significantly delayed CRPC progression [57].

Another mechanism of resistance to enzalutamide is mediated by NF-kB2/p52 via activation of AR and its splice variants. Nadiminty et al. demonstrated that PC cells chronically treated with enzalutamide exhibit higher levels of NF-kB2/p52 and enhanced expression of ARV7 whereas expression of FL AR remained unchanged. Conversely *in vitro* down-regulation of NF-kB2/p52 in enzalutamide treated PC cells reduced ARVs expression and made cells more sensitive to enzalutamide [58].

Neuroendocrine transformation

Neuroendocrine carcinoma of the prostate represents a subset of PC phenotypes that may be linked to resistance to AR signaling inhibition, aggressive tumor characteristics and poor prognosis. Recently, it was shown that treatment with enzalutamide may cause a reduced expression of the repressor element 1 silencing transcription factor (REST), a mediator of AR action on gene repression that may be responsible of the neuroendocrine differentiation [59]. Neuroendocrine transformation may partly explain cross-resistance between abiraterone and enzalutamide.

Autophagy

Autophagy is a tightly regulated catabolic process of cellular self-digestion by which cellular components are targeted to lysosomes for their degradation. Different forms of autophagy can be identified including macro-autophagy, micro-autophagy and chaperone-mediated autophagy.

Macro-autophagy is associated with drug resistance in different types of cancer and can acts as an adaptive response to maintain cell survival under metabolic stresses, including androgen deprivation [60]. Recent studies suggested that autophagy has a pro-survival role in androgen-responsive cell lines LNCaP treated with androgen deprivation [61].

In a recent study Nguyen et al. showed that enzalutamide induces autophagy in androgen dependent CRPC cell lines by AMPK activation and mTOR suppression. Furthermore they demonstrated that small interfering RNA targeting AMPK significantly inhibited autophagy and promoted cell death in CaP cells treated with enzalutamide, suggesting that autophagy is an important survival mechanism in CRPC [62]. Finally, *in vivo* studies in orthotopically implanted mice with enzalutamide-resistant cells showed that the combination of enzalutamide and the autophagy modulators clomipramine or metformin significantly reduced tumor growth compared to control ($p < 0.005$) [62].

For chaperone-mediated autophagy, Matsumoto et al. reported that enzalutamide induced activation of clusterin, a cytoprotective chaperone, and clusterin knockdown synergically enhanced enzalutamide-induced inhibition of LNCaP cell growth. The co-inhibition of the AR (with enzalutamide) and clusterin (with OGX-011) synergically enhanced apoptotic rates and delayed CRPC LNCaP tumor and PSA progression *in vivo* [63].

Immune evasion: PD-L1/PD-1 up-regulation

Bishop et al. demonstrated in blood of patients progressing during treatment with enzalutamide a significant increase of PD-L1/2-positive dendritic cells (DC) compared to treatment naïve or responsive patients, and a correlation with treatment duration and poorer initial response to enzalutamide was also shown. These data are aligned with preclinical findings indicating increased amounts of circulating PD-L1/2 + DC in mice with enzalutamide-resistant tumors compared to CRPC, and these resistant tumors also expressed significantly increased levels of tumor-intrinsic PD-L1 [64].

Discussion

In the 1990s dedicated studies on AR in CRPC revealed escape mechanisms from androgen blockade through either mutation or amplification of AR [14]. Additionally, these resistance mechanisms corroborate the hypothesis that androgen-signaling remains biologically relevant despite castrate levels of testosterone and these observations guided the research and development of a new generation of androgen synthesis inhibitors and AR antagonists [4] and [5].

However, primary and acquired resistance to new hormonal agents approved for the treatment of CRPC is a complex phenomenon, not completely understood, involving AR signaling pathway and also dependant from AR bypassing mechanisms. In preclinical studies resistance to abiraterone and enzalutamide is mediated by similar mechanisms, including steroidogenesis up-regulation and AR overexpression (Table 2) [29].

Abiraterone induces androgen accumulation, such as pregnenolone and progesterone, upstream to the CYP17A1 enzymatic block. It has been demonstrated that mCRPC patients have an increased expression of PR [65] and that PR high density in PC cells is an independent poor prognostic factor [66]. PR and AR have 88% sequence homology in the ligand-binding domain and share response elements in multiple gene targets [67]. As a consequence the progesterone binding to PR can induce transcription of androgen dependent genes. In a phase 1–2 study Jajaram et al. showed that progesterone receptor inhibition by onapristone, a progesterone receptor antagonist, in patients with CRPC progressed after abiraterone, enzalutamide or two lines of chemotherapy is feasible and safe [68]. This trial is ongoing and activity data are still pending (Table 3).

Table 3.

Proposed strategies to overcome abiraterone and/or enzalutamide mechanism of resistance and ongoing clinical trial.

Mechanism of resistance	How to overcome it	Ongoing clinical trials
• AR overexpression	Enzalutamide in combination with abiraterone acetate	NCT01650194: Phase 2
	Increased-dose abiraterone acetate (2000 mg daily)	NCT01637402: Phase 2
• Androgen biosynthesis pathway upregulation	ARN-509 + abiraterone	NCT02123758: Phase 1
	ARN-509 + abiraterone	NCT01792687: Phase 1
	ARN-509 + abiraterone	NCT02257736: Phase 3
• Intracrine androgen synthesis	Galeterone (AR antagonist and CYP17A1 inhibitor)	NCT01709734: Phase 2
	Onapristone (progesterone receptor inhibitor)	NCT02049190: Phase1/2
• Immune evasion	Pembrolizumab (anti PD-1) following Enzalutamide	NCT02312557: Phase 2
	PROSTVAC (vaccine therapy) + enzalutamide	NCT01867333
• ARVs	EPI-506 (NTD-binding AR antagonist)	NCT: Pending FDA approval
• Autophagy	OGX-427 + abiraterone	NCT01681433: Phase 2
	Alisertib (selective aurora A kinase	NCT01848067:

Mechanism of resistance	How to overcome it	Ongoing clinical trials
induction	inhibitor) + abiraterone	Phase 1/2
	AT 13387 (HSP90 inhibitor) + abiraterone	NCT01685268: Phase 1/2
• Activation of other pathways	Crizotinib + enzalutamide	NCT02207504: Phase 1
	Abiraterone + BEZ235 (Pi3K and mTOR inhibitor) or Abiraterone + BKM120 (Pi3K inhibitor)	NCT01634061: Phase 1
	Dovitinib (multitargeted TKI) + Abiraterone	NCT01994590: Phase 2
	Dasatibib (mutitargeted TKI) + abiraterone	NCT01685125: Phase 2
	Olaparib (PARP-inhibitor) + abiraterone	NCT01972217: Phase 2
	Everolimus (m-TOR inhibitor) + ARN-509 (AR antagonist)	NCT02106507: Phase 1
	BI 836845 (anti IGF1/IGF2) + enzalutamide	NCT02204072: Phase 1
	Cabozantinib (anti c-MET and anti VEGFR2) + abiraterone	NCT01574937: Phase 1
• Other mechanisms	Metformin + abiraterone	NCT01677897: Phase 2
	Metformin + enzalutamide	NCT02339168: Phase 1
	Cabazitaxel + abiraterone	NCT02218606: Phase 2

Table options

Progesterone can also stimulate AR in presence of specific AR LBD mutations and can be converted to DHT via backdoor pathway [17].

Moreover, abiraterone does not completely block serum precursor steroids such as DHEA-S that can be transported and metabolized in cancer cells to DEA, a steroid downstream of CYP17A1, bypassing the enzymatic block induced by abiraterone [29].

When CYP17A1 is inhibited, 3β-hydroxysteroid dehydrogenase becomes the main enzyme involved in steroid synthesis. It is responsible of progesterone synthesis from pregnenolone, androstenedione synthesis from DHEA and TST synthesis from androstenediol (Fig. 1). *In vitro* studies showed that abiraterone, when administered at high dose, is able to inhibit both 3β-hydroxysteroid dehydrogenase activity and AR activity [31]. An ongoing trial is investigating higher vs standard doses of abiraterone for the treatment in CRPC patients (Table 3).

The AR signaling pathway reactivation as resistance mechanism to abiraterone and increased androgen synthesis in patients treated with enzalutamide represent the biological background to test the combination of abiraterone with new generation AR antagonists, such as enzalutamide and ARN-509. An ongoing phase II study is administering simultaneously abiraterone and enzalutamide to CRPC patients with bone metastases. Preliminary results from this study indicate that the combination has a favorable safety profile and 48% of patients experienced PSA decline $\geqslant$90% and 72% a PSA decline $\geqslant$50% [69] (Table 3).

ARN-509 is a new AR antagonist that in xenograft models binds to AR with a superior potency than enzalutamide [70]. Several ongoing trials are evaluating the combination of abiraterone with enzalutamide or ARN-509 (Table 3).

Sharing the same background, an ongoing phase III trial is investigating the efficacy of galeterone in chemotherapy-naïve CRPC patients (Table 2). Galeterone is an innovative hormonal agent that, at low concentrations, inhibits CYP17 activity, while at high concentrations induces AR degradation [71]. Due to its ability to induce AR degradation, galeterone could be active also in presence of AR splice variants.

One of the most important resistance mechanisms is related to the detection of AR splicing variants and in presence of LBD loss of function NTD becomes the main AR domain, being crucial for transcriptional AR activity [40]. EPI-001 is a small molecule in preclinical development that binds the AR-transactivation domain of NTD causing the block of interaction of AR with AREs. In xenograft models EPI-001 demonstrated efficacy in inhibiting the growth of CRPC models without causing toxicity [72]. The unique mechanism of action of this drug suggests that it can be active in presence of AR splicing variants. An ongoing phase 1–2 trial in men with enzalutamide and/or abiraterone-failure mCRPC, is evaluating the safety and antitumor activity of EPI-506, another NTD inhibitor. (Table 3).

Resistance mechanisms to abiraterone and enzalutamide are peculiar and often different from those induced by chemotherapy. Recently Antonarakis assessed the correlation between the detection of ARV-7 on circulating tumor cells and response in 37 CRPC patients treated with docetaxel or cabazitaxel. PSA response in ARV-7-positive and in ARV-7-negative patients was comparable (41% vs 65%, $P = 0.19$) as well as median PFS (5.1 vs 6.9 months, HR 2.65, $P = 0.11$) [48] and [73]. Taken together these data indicate that the combination of new hormonal agents with taxanes should be further investigated in the attempt to kill both ARV-7 positive and negative cancer cells and consequently to delay the onset of resistant clones [74]. Rationale for concomitant administration of new generation hormonal therapies and taxanes relies on advanced prostate carcinoma biology. Such disease, indeed, is heterogeneous with AR positive cells coexisting with cells not depending from AR pathway, the latter in principle sensitive to chemotherapy. Considering these biological basis and the above mentioned data it is reasonable to hypothesize that the concomitant administration of new hormonal agents and taxanes can be more advantageous in terms of outcome as compared to the sequential administration. An ongoing randomized phase II trial is assessing the combination of cabazitaxel and abiraterone in metastatic CRPC, compared to cabazitaxel alone, with progression-free survival after 3 months as primary endpoint (ClinicalTrials.gov Identifier NCT01845792). However, beside the risk of increased toxicity, it should be underlined that a combinatory approach has the potential risk to “spend” two effective agents in the same treatment line. A clinical trial adequately designed to compare the efficacy of the association of cabazitaxel and abiraterone or enzalutamide vs the sequential administration of those agents is warranted. In such a trial, comparing two different treatment strategies (combination vs sequential approach), the adequate endpoint would probably be overall survival, and not the progression-free survival of a single line of treatment.

Heat shock proteins (HSPs) inhibitors represent another treatment opportunity to overcome acquired resistance to hormonal therapies. These agents are cytoprotective chaperones crucial for cytoplasmic AR stabilization [75]. In particular HSP27 acts as anti-apoptotic molecule and is overexpressed in CRPC patients while HSP90 promotes the ligand-independent nuclear translocation of AR [75]. A study of AT13387, a potent inhibitor of HSP90, in combination with abiraterone has recently been completed. Another phase II trial is assessing the efficacy of abiraterone in association with an HSP27 inhibitor OGX-427 in CRPC patients with progressing levels of serum PSA (Table 3).

There is a strong rationale to combine new generation hormonal therapies with biologic drugs. The inhibition of AR signaling activates several oncogenic pathways implicated in cancer cell survival, being the most important the Pi3K-AKT-mTOR and the MET signaling pathways. Initial trials are investigating the activity of abiraterone in combination with everolimus, an mTOR inhibitor, or BKM120, a Pi3K inhibitor and BEZ235 a dual Pi3K/mTOR inhibitor (Table 3).

MET overexpression has been observed in primary and metastatic prostate carcinoma [76]. In xenograft models Verras et al. showed that castration induces c-Met expression and AR represses c-Met expression directly through binding to its promoter [77]. Cabozantinib is a small molecule kinase inhibitor with potent activity against MET and VEGFR2. Preliminary data of a phase 1 study showed that 20 or 40 mg of cabozantinib is feasible and safe when combined with Abiraterone [78]. The phase 2 trial testing the

combination of cabozantinib and abiraterone was stopped after results of cabozantinib Phase 3 CRPC study XL184–307. Several ongoing trials are evaluating the combination of abiraterone or enzalutamide with agents acting on AR-independent pathways such as crizotinib, a mesenchymal-epithelial transition (MET)/ALK multi-targeted receptor tyrosine kinase inhibitor, dasatinib, a src inhibitor, dovitinib, a multi-targeted receptor tyrosine kinase inhibitor, olaparib and veliparib, two PARP inhibitors. Alisertib, a selective aurora A kinase inhibitor and immunotherapies such as pembrolizumab (anti-PD1) and PROSTVAC (vaccine therapy) are other agent currently investigated in this setting (Table 3).

Conclusion

Although during the past decade specific molecular alterations have been identified in the so called "oncogene addicted" tumors that led to the development of specific and effective targeted therapies that induce tumor shrinkage in most of the treated cases as well as significantly extend progression-free survival, the responses to these agents is limited by the onset of acquired resistance. A deeper understanding of resistance mechanisms to these drugs is essential to maximize their efficacy and develop new strategies to overcome them.

New generation hormonal therapies in CRPC, such as enzalutamide and abiraterone, have demonstrated also to prolong overall survival, but a proportion of patients do not benefit from these treatments and most patients develop resistance within a year. Primary and acquired resistance to abiraterone and enzalutamide depend from both AR-dependent or AR-independent mechanisms. Several novel agents, including new AR inhibitors, androgen synthesis inhibitors, HSP modulators, immunotherapies, tyrosine kinase inhibitors and others, are currently in clinical testing to prevent or overcome these resistance mechanisms. The combination of these drugs and hormonal therapies may synergically act to improve the clinical activity of systemic therapy in CRPC.

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