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Current advances in intratumoral androgen metabolism in castration-resistant prostate cancer

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Abstract

Purpose of review—Androgen deprivation therapy is a cornerstone in the treatment of advanced prostate cancer and has extended the lives of countless patients. Unfortunately, many of these patients eventually succumb to metastatic castration-resistant prostate cancer (mCRPC). The efficacy of abiraterone acetate (Zytiga) and enzalutamide (Enza, Xtandi) in the mCRPC setting prove that these tumors remain androgen-driven. We review recent studies that have shown that intratumoral androgen biosynthesis plays a significant role in the ever-evolving mCRPC tumor and we discuss the therapeutic implications of these findings.

Recent findings—A novel abiraterone metabolite, D4A, possesses robust anti-tumor activity in rodent models via the inhibition of androgen biosynthetic enzymes and antagonism of AR. The TMPRSS2:ERG fusion drives AKR1C3 expression and activity to facilitate androgen biosynthesis and activate the androgen receptor (AR) in prostate cancer. Intracrine androgen formation and AKR1C3 expression and activity have been found to confer resistance to enzalutamide.

Summary—These studies highlight the significant role that intratumoral androgen biosynthesis plays in the mCRPC tumor. The therapeutic implications include the inhibition of AKR1C3 in tumors that become resistant to current drugs such as AA or Enza and the potential administration of D4A as a mCRPC therapeutic.

Keywords

Metastatic castrate-resistant prostate cancer; androgen metabolism; abiraterone acetate; enzalutamide; AKR1C3

Introduction

The legacy of Huggins and Hodges' landmark discovery of the use of surgical castration or orchiectomy and adrenalectomy to treat advanced prostate cancer continues to this day. Androgen deprivation is achieved in the clinic using first line therapies such as leuprolide

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Conflict of Interest

TMP is the founder of Penzymes, LLC.

(an LH-RH agonist), *R*-bicalutamide (an AR antagonist) and dutasteride (dual 5 α -reductase type 1 and type 2 inhibitor). Second line therapies include abiraterone acetate (AA), which inhibits cytochrome P450 17 α -hydroxylase, 17,20-lyase (CYP17A1) in the adrenal glands and in the tumor, and enzalutamide (Enza), which acts as an AR super-antagonist, inhibits AR translocation to the nucleus and increases AR degradation. New agents are in development and include the cytochrome P450 17,20-lyase specific inhibitor, galeterone (TOK-001) [1] and the next-generation AR antagonists such as apalutamide (ARN-509) [2], ODM-201 [3], EPI-001 [4] and BMS-641988 [5], some of which have been shown to reduce specific side-effects of existing drugs while maintaining or improving upon the intensity of androgen deprivation or AR antagonism. The androgen deprivation therapy (ADT) agents are complemented with drugs that work via non-androgen signaling pathways such as the immunotherapeutic, sipuleucel-T (Provenge) [6], radiation therapy [7, 8] and the taxanes such as docetaxel and cabazitaxel [9–11]. The growing number of therapeutic choices has led to the question of the sequence with which to administer the therapies and the need for robust biomarkers in order to personalize treatment regimens. Perhaps the most pressing issue in prostate cancer is the need to surmount castration resistance, drug resistance or the combination of the two.

The underlying biology that drives metastatic castration-resistant prostate cancer (mCRPC) progression is complex. As the field elucidates the mechanisms that underpin mCRPC, the treatment regimen can be personalized to the individual by validating and using biomarkers to guide the decision as to which drug to administer to increase patient survival. For example, the AR splice variant, AR-V7, is an area of intense interest. The ligand-binding domain is deleted in AR-V7, resulting in a constitutively active AR. The dynamics of the emergence of AR-V7 in circulating tumor cells (CTCs) is of particular interest [12] as it may be predictive of non-responders to AA and Enza. Therefore, AR-V7 positive patients may be candidates for radiation or taxane treatment [13], while AR-V7 negative patients may be treated with ADT. However, the AR-V7 does not account for all of the non-responding tumors and there is abundant evidence to suggest that aberrations in the pre-receptor biology may also be a mechanism underlying progression to mCRPC and drug resistance [14–16]. Here, we highlight recent studies that have interrogated the pre-receptor biology and give insight into putative therapeutic targets for mCRPC. The first study examines the properties of D4A, a novel metabolite of abiraterone that results from its metabolism via 3 β -hydroxysteroid dehydrogenase (3 β -HSD) type 1. Then, we examine two recent studies that have added to the body of work on the significant role that the androgen biosynthetic enzyme aldo-keto reductase 1C3 (AKR1C3), also known as 17 β -hydroxysteroid dehydrogenase type 5 (17 β -HSD5), plays in mCRPC biology.

Text of Review

The second line ADT therapies, AA and Enza are clinically approved for use in the mCRPC setting. AA has extended the lives of patients who have progressed after ADT and docetaxel treatment by a median of 1–2 months [17]. This finding was significant because it established that mCRPC tumors remain androgen driven. However, the clinical experience has shown that the tumor ultimately evolves to become resistant to AA. Clinical studies have shown the efficacy of AA in the neoadjuvant space and this may improve outcomes [18].

Enza has also been shown to extend median survival in the post-ADT and post-docetaxel mCRPC space by a median of 1–2 months [19] and was found to significantly improve survival relative to *R*-bicalutamide [20, 21]. Here, we critically review several topical studies on the role of androgen metabolism in mCRPC biology and the ramifications of their findings on the treatment of mCRPC.

D4A, a novel metabolite of AA

In a study by Li and colleagues [22], Abiraterone was found to be a substrate for metabolism by 3 β -HSD type 1 to form a 4-3-ketosteroid metabolite of abiraterone which was referred to as D4A. This novel metabolite is of minor abundance in human patient serum, comprising on average, approximately 4% of total AA after a 1000 mg dose. The inhibition of CYP17A1 and 3 β -HSD1 by D4A is comparable to that seen with abiraterone. This raises the question as to whether abiraterone acts as an inhibitor of 3 β -HSD1 or whether it exerts its effect after conversion to the D4A metabolite. D4A was also found to inhibit 5 α -reductase activity, whereas abiraterone did not. Furthermore, D4A was found to have increased antagonism towards AR relative to abiraterone (Figure 1). In LNCaP cells, D4A inhibited gene expression of PSA, TMPRSS2 and FKBP5 upon stimulation with DHEA, DHT and R1881. These findings indicate that the D4A metabolite is bi-functional and mediates its effects by inhibition of androgen biosynthesis and by AR antagonism. D4A inhibited DHT-induced proliferation of LNCaP cells. In LNCaP or VCaP xenografts in the NSG mouse, the adrenal glands express 3 β HSD-1 and were thus the site at which D4A was produced, i.e. D4A was not produced in the mouse prostate tissue. The study also showed that D4A exhibited improved progression-free survival in both VCaP and C4-2 rodent xenograft models relative to AA. In the VCaP xenograft model D4A inhibited growth significantly better than AA at 3 days ($p < 0.01$) and at 9 days ($p < 0.05$) [22].

These findings present a case for the administration of the D4A metabolite in prostate cancer therapy. The main advantage of the D4A compound as highlighted by the molecular studies is the added benefit of 5 α -reductase inhibition and AR antagonism. The efficacy of D4A was well-characterized by Li and colleagues using a variety of approaches. It is of interest that much of the molecular work was done in the LNCaP cell line and the rodent xenograft model employed the VCaP and C4-2 cell lines. The LNCaP cell line has a mutated AR (T877A), suggesting that D4A can also antagonize the mutant AR. Thus far, the D4A metabolite has yet to be screened as a ligand for other steroid receptors or tested for efficacy in patient derived xenografts. On a broader level, the findings present a paradox, whereby previous findings by KH Chang and colleagues showed that the N376T mutant of 3 β -HSD1 facilitated the stabilization of the protein and biosynthesis of DHT in the mCRPC tumor and was therefore postulated to be a poor prognostic indicator [23]. In light of this study, patients with the N376T mutant tumors should be expected to generate more D4A metabolite intratumorally and patients harboring this mutation should have an improved response to abiraterone.

The ERG/AKR1C3/AR androgen axis

In prostate cancer, the TMPRSS2:ERG gene fusion has been shown to be over-expressed in higher Gleason grade disease and at metastatic sites. However, the exact role of ERG has not

been clearly examined. In a study by Powell and colleagues, VCaP cells were used to establish a lentiviral shRNA knockdown of ERG [24]. In the VCaP shERG knockdown cells, transcripts of AKR1C3, 17 β -HSD6 and 17 β -HSD4 were significantly reduced relative to the VCaP-sh-scrambled control. This correlation was also seen with AKR1C3 protein expression levels. In LNCaP and BH-1 cells, the over-expression of ERG-40 (a truncated version of ERG lacking the first 39 a.a. on the N-terminus) resulted in the up-regulation of AKR1C3. CHIP-seq data of VCaP cells identified two binding peaks far upstream of the transcriptional start site (TSS) in the AKR1C3 gene, one at ~50 kb, one at ~75 kb and another one in intron 1. In VCaP-shERG cells, the conversion of 5 α -androstane-3,17-dione (Adione) to DHT was reduced by ~80% relative to the VCaP-sh-scrambled control. VCaP cells supplemented with Adione led to the translocation of AR and stimulated PSA expression. However, PSA expression was diminished in VCaP-shERG and VCaP-shAKR1C3 relative to controls (Figure 2). The study used bioinformatics to probe the relationship between ERG and AKR1C3 in patient samples. In a cohort of 64 patients, 24 were ERG⁺/AKR1C3⁺ and 25 were ERG⁻/AKR1C3⁻. Furthermore, Oncomine, which is a cancer microarray database and data mining platform (<http://www.oncomine.org>) was used to query for TMPRSS2:ERG and AKR1C3 expression in prostate cancer tumors and generate Kaplan-Meier curves. This analysis showed poorer survival for prostate cancer patients with tumors that had TMPRSS2:ERG⁺/high AKR1C3 expressing tumors relative to patients that had TMPRSS2:ERG⁻/low AKR1C3 expressing tumors [24].

This study shows that ERG exerts regulatory control over the expression and activity of AKR1C3, 17 β -HSD6 and 17 β -HSD4. 17 β -HSD4 is an NAD⁺-dependent dehydrogenase that inactivates T and DHT by converting them to 4-AD and Adione, respectively [25]. The regulation of 17 β -HSD6 is extremely interesting in the context of intratumoral androgen metabolism as it is the predominant 3 α -HSD responsible for the conversion of 3 α -Adiol to DHT via the “backdoor pathway” [26]. Moreover, in VCaP shERG knockdown cells, a decreased conversion of Adione to DHT by AKR1C3 and diminished downstream AR translocation and PSA expression were observed. These findings strongly support an ERG/AKR1C3/AR feed-forward signaling mechanism. In this mechanism, TMPRSS2:ERG is expressed in advanced disease, overriding the repressive effect of AR on the AKR1C3 promoter. Increased production of T and DHT by AKR1C3 induces TMPRSS2:ERG, leading to the feed-forward up-regulation of AKR1C3. The bioinformatics work also displays a correlation between ERG and AKR1C3 expression in patient samples. Furthermore, patient prognosis is worse in patients that are positive for the TMPRSS2:ERG gene fusion and have elevated AKR1C3 expression, relative to those that do not possess the gene fusion and have low AKR1C3 expression. This would seem to be at odds with recent clinical studies which have found that the TMPRSS2:ERG fusion predicts for tumors that are more responsive to ADT, though these studies did not look at AKR1C3 expression levels [27, 28].

Intracrine androgens and AKR1C3 confers resistance to enzalutamide

The AR super-antagonist, Enza, has become one of the mainstays in the clinicians ADT tool kit. Unfortunately, resistance to Enza is a significant problem that ultimately results in patient death. In this study, Liu and colleagues developed an Enza-resistant sub-line of the

C4-2B cell line after serial exposure to the drug [29]. They called this sub-line, C4-2B MDVR. The C4-2B MDVR cells were more resistant to enzalutamide relative to the parental C4-2B cells in an orthotopic SCID mouse. The transcript level and protein expression of androgen biosynthetic enzymes such as 3 β -HSD1, AKR1C3 and CYP17A1 were up-regulated in the C4-2B MDVR cells relative to the parental C4-2B cells (Figure 3). AKR1C3 protein expression levels were measured in a panel of prostate cancer cell lines and were as follows: VCaP > CWR22Rv1 > C4-2B MDVR > LN-95 > LNCaP > C4-2B. Using Oncomine, the study found AKR1C3 to be low in benign prostate and primary prostate cancer and elevated in metastatic prostate cancer. Furthermore, the Oncomine data showed that AKR1C3 expression levels correlate with Gleason score and recurrence. Quantification of select androgens showed that T, DHT and DHEA were higher in C4-2B MDVR cells vs. C4-2B cells. Knockdown of AKR1C3 in the C4-2B MDVR and CWR22Rv1 cell lines sensitized these cells to Enza treatment. The converse approach was used and the over-expression of AKR1C3 in LNCaP cells conferred resistance to Enza relative to the parental LNCaP cells. The non-steroidal anti-inflammatory drug (NSAID), indomethacin, is a potent and selective AKR1C3 inhibitor identified by our lab [30], and was used alone and in combination with Enza in this study. In clonogenic assays with C4-2B MDVR and CWR22Rv1 cells, the combination of indomethacin and Enza reduced colony formation by ~70–80%, with the more pronounced effect in C4-2B MDVR cells compared to CWR22Rv1 cells. In the CWR22Rv1 xenograft model, indomethacin alone had a more pronounced effect on tumor volume reduction compared to Enza alone. The most robust response in tumor volume reduction and Ki67 staining came from the combination of indomethacin and Enza [29].

This study shows for the first time that AKR1C3 along with several other androgen biosynthetic enzymes are over-expressed as a result of long-term Enza treatment of the C4-2B prostate cancer cell line. The C4-2B MDVR cells were sensitized to Enza using indomethacin in clonogenic assays and in a xenograft model using the CWR22Rv1 cell line; pointing the way to how Enza-resistance may be treated clinically. The data provides a compelling case that AKR1C3 may be a therapeutic target in Enza-resistant mCRPC.

Targeting intratumoral androgen metabolism in mCRPC

Improving the survival of mCRPC patients is an area of unmet need and tremendous opportunity. The second-line therapies, AA and Enza have extended the survival in many patients that become refractory to first-line therapies. However, the eventual emergence of AA- or Enza-resistance still claims the lives of the vast majority of these patients. The administration of these drugs in the neo-adjuvant setting [18] and the up-front combination of ADT with taxanes [9] has begun to yield improved results. Indeed, clinical evidence is beginning to emerge that suggests that more intense and continuous androgen deprivation correlates with improved outcomes [31]. The interrogation of the alterations in androgen biosynthetic enzymes that occur in the tumor during progression to mCRPC have given us insight into putative therapeutic targets. The discovery and characterization of the D4A metabolite by Li and colleagues is of interest [22] as it dovetails with the data from Liu and colleagues which found that long-term Enza treatment resulted in the up-regulation of a panel of androgen biosynthetic enzymes including AKR1C3, 3 β -HSD1 and CYP17A1 [29].

The two studies taken together would suggest that a subpopulation of Enza-resistant patients that have not yet been treated with AA may over-express 3 β -HSD1, which would generate the D4A metabolite intratumorally and may be patients who stand to benefit from AA therapy. These tumors may also be candidates for AKR1C3 inhibition.

AKR1C3 is over-expressed in prostate tumors relative to normal prostate tissue and in response to ADT. Castration of a VCaP orthotopic model has been found to lead to the up-regulation of steroidogenic enzymes including AKR1C3 [32]. Our lab has been at the forefront in elucidating the role of AKR1C3 in prostate cancer [33]. We have developed lead drug candidates for the specific inhibition of AKR1C3 over the AKR1C1 and AKR1C2 isoforms whose activity is desirable because they inactivate the potent AR ligand, DHT [34, 35]. Our work with clinical collaborators has yielded data from the neo-adjuvant leuprolide/AA clinical trial that supports the hypothesis that the DHEA sulfate depot that remains post-AA may feed intratumoral androgen biosynthesis. As AKR1C3 is one of the most overexpressed steroidogenic genes in CRPC, its presence plus the DHEA sulfate pool could create a perfect-storm for AA resistance [36]. Thus inhibition of AKR1C3 would be desirable. While the role of AKR1C3 in intratumoral androgen biosynthesis is a primary focus with regards to prostate cancer, the inhibition of AKR1C3 may also exert beneficial anti-proliferative effects via inhibition of either its prostaglandin F synthase activity or its AR coactivator function or its role in regulating AR transcriptional activity via the inhibition of Siah2 self-ubiquitination [37–39]. Furthermore, the metastases of prostate cancer to the bone may be facilitated by the overexpression of AKR1C3 among other steroidogenic enzymes [40].

Conclusion

The treatment of prostate cancer has evolved considerably since the days of Huggins and Hodges and patient outcomes have improved. However, the emergence of mCRPC and drug resistance is the biggest hurdle to improving patient survival. Androgen biosynthetic enzymes play an integral role in the mCRPC tumor and in an Enza resistance model. This sets up a potential therapeutic strategy of targeting these key players such as AKR1C3 in order to re-sensitize these tumors to the current ADT armamentarium.

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References

1. Montgomery B, Eisenberger MA, Rettig MB, et al. Androgen receptor modulation optimized for response (ARMOR) phase I and II studies: galeterone for the treatment of castration-resistant prostate cancer. Clin Cancer Res. 2015 [E-pub ahead of print]. Phase I/II trials showed that galeterone, a selective CYP17 inhibitor and AR degrader, was well-tolerated and efficacious for the treatment of naïve non-metastatic and mCRPC.
2. Rathkopf DE, Morris MJ, Fox JJ, et al. Phase I study of ARN-509, a novel antiandrogen, in the treatment of castration-resistant prostate cancer. J Clin Oncol. 2013; 31(28):3525–3530. [PubMed: 24002508]

3. Fizazi K, Massard C, Bono P, et al. Activity and safety of ODM-201 in patients with progressive metastatic castration-resistant prostate cancer (ARADES): an open-label phase 1 dose-escalation and randomized phase 2 dose expansion trial. *Lancet Oncol.* 2014; 15(9):975–985. [PubMed: 24974051] In this Phase I trial, ODM-201, an AR antagonist that prevents AR translocation, showed disease suppression and a favorable safety profile when administered to patients with progressive mCRPC.
4. Brand LJ, Olson ME, Ravindranathan P, et al. EPI-001 is a selective peroxisome proliferator-activated receptor-gamma modulator with inhibitory effects on androgen receptor expression and activity in prostate cancer. *Oncotarget.* 2015; 6(6):3811–3824. [PubMed: 25669987] This study examined EPI-001, an N-terminal domain inhibitor of AR, raised some issues as to off-target effects that include PPAR- γ activation and reactivity with cellular thiols.
5. Balog A, Rampulla R, Martin GS, et al. Discovery of BMS-641988, a novel androgen receptor antagonist for the treatment of prostate cancer. *ACS Med Chem Lett.* 2015; 6(8):908–912. [PubMed: 26288692] This study tested the binding affinity and efficacy of BMS-641988 in several human prostate cancer xenograft models.
6. Small EJ, Raymond L, Gardner TA, et al. A randomized phase II trial of sipuleucel-T with concurrent vs sequential abiraterone acetate plus prednisone in metastatic castration resistant prostate cancer. *Clin Cancer Res.* 2015; 21(17):3862–3869. [PubMed: 25925891] This clinical trial established that sipuleucel-T manufacture can be accomplished concurrent with AA and prednisone treatment without affecting immunologic effects or raising any safety issues.
7. Mason MD, Parulekar WR, Sydes MR, et al. Final report of the intergroup randomized study of combined androgen-deprivation therapy plus radiotherapy versus androgen-deprivation therapy alone in locally advanced prostate cancer. *J Clin Oncol.* 2015; 33(19):2143–2150. [PubMed: 25691677] This clinical trial of 1,205 patients with locally advanced prostate cancer showed the benefit of combined ADT with radiotherapy at a median follow-up of 8 years.
8. D'Amico AV, Chen MH, Renshaw A, et al. Long-term follow-up of a randomized trial of radiation with or without androgen deprivation therapy for localized prostate cancer. *JAMA.* 2015; 314(12):1291–1293. [PubMed: 26393854] This clinical trial of 206 men with unfavorable-risk prostate cancer showed a benefit of combined ADT with radiation therapy vs radiation therapy alone at a median follow-up of 10–12 years. However, radiation therapy alone was found to have fewer deaths due to cardiac events in patients with moderate or severe comorbidity relative to ADT with radiation therapy.
9. Sweeney CJ, Chen YH, Carducci M, et al. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. *N Engl J Med.* 2015; 373(8):737–746. [PubMed: 26244877] This clinical trial showed that concurrent docetaxel treatment with ADT resulted in significantly longer overall survival in patients with metastatic, hormone-sensitive prostate cancer.
10. James ND, Sydes MR, Clarke NW, et al. Addition of docetaxel, zoledronic acid, or both to first-line long-term hormone therapy in prostate cancer (STAMPEDE): survival results from an adaptive, multiarm, multistage platform randomized controlled trial. *Lancet.* 2015 [E-pub ahead of print]. This clinical trial showed that docetaxel treatment given at the initiation of long-term first line hormone therapy improved survival but also increases some adverse drug events.
11. van Soest RJ, Nieuweboer AJ, de Moree ES, et al. The influence of prior novel androgen receptor targeted therapy on the efficacy of cabazitaxel in men with metastatic castration-resistant prostate cancer. *Eur J Cancer.* 2015; 51(17):2562–2569. [PubMed: 26278646] This clinical trial investigated the effect of prior ADT on the efficacy of cabazitaxel in mCRPC patients. In a cohort of 114 patients, this study showed that prior ADT did not have an effect on the efficacy of cabazitaxel.
12. Nakazawa M, Lu C, Chen Y, et al. Serial blood-based analysis of AR-V7 in men with advanced prostate cancer. *Ann Oncol.* 2015; 26(9):1859–1865. [PubMed: 26117829] This study of 14 patients with metastatic prostate cancer were assessed for the dynamics of AR-V7 status during the course of therapy. AR-V7 negative CTCs were found to convert to AR-V7 positive during ADT and taxane therapies, however, AR-V7 positive CTCs were found to revert to AR-V7 negative only during taxane therapy.
13. Antonarakis ES, Lu C, Luber B, et al. Androgen receptor splice variant 7 and efficacy of taxane chemotherapy in patients with metastatic castration-resistant prostate cancer. *JAMA Oncol.* 2015;

- 1(5):582–591. [PubMed: 26181238] This study showed that AR-V7 detected in circulating tumor cells from CRPC patients is predictive for tumors that are resistant to AA and Enza.
14. Zhang A, Zhang J, Plymate S, et al. Classical and non-classical roles for pre-receptor control of DHT metabolism in prostate cancer progression. *Horm Cancer*. 2016 [E-pub ahead of print]. This review summarizes the current body of knowledge on the role of androgen biosynthetic enzymes in play in the primary prostate tumor and the CRPC tumor. They also use recent data to set forth non-canonical roles for these enzymes.
 15. Penning TM. Mechanisms of drug resistance that target the androgen axis in castration resistant prostate cancer (CRPC). *J Steroid Biochem Mol Biol*. 2015; 153:105–113. [PubMed: 26032458] This review examines the adaptive mechanisms that the CRPC tumor deploys to become resistant to the current ADT armamentarium. Mechanisms include alterations in the pre-receptor androgen biosynthetic enzymes and alteration in the AR itself.
 16. Labrie F. Combined blockade of testicular and locally made androgens in prostate cancer: a highly significant medical progress based upon intracrinology. *J Steroid Biochem Mol Biol*. 2015; 145:144–156. [PubMed: 24925260] This review examines the data that shows that intracrinology or the formation of intraprostatic androgens is a driver of CRPC.
 17. Fizazi K, Scher HI, Molina A, et al. Abiraterone acetate for treatment of metastatic castration-resistant prostate cancer: final overall survival analysis of the COU-AA-301 randomised, double-blind, placebo-controlled phase 3 study. *Lancet Oncol*. 2012; 13(10):983–992. [PubMed: 22995653]
 18. Taplin ME, Montgomery B, Logothetis CJ, et al. Intense androgen-deprivation therapy with abiraterone acetate plus leuprolide acetate in patients with localized high-risk prostate cancer: results of a randomized phase II neoadjuvant study. *J Clin Oncol*. 2014; 32(33):3705–3715. [PubMed: 25311217] This clinical trial showed intensive suppression of intratumoral androgens when localized high-risk prostate cancer patients were given leuprolide + AA + prednisone, and was much more effective than leuprolide alone.
 19. Scher HI, Fizazi K, Saad F, et al. Increased survival with enzalutamide in prostate cancer after chemotherapy. *N Engl J Med*. 2012; 367(13):1187–1197. [PubMed: 22894553]
 20. Shore ND, Chowdhury S, Villers A, et al. Efficacy and safety of enzalutamide versus bicalutamide for patients with metastatic prostate cancer (TERRAIN): a randomized double-blind, phase 2 study. *Lancet Oncol*. 2016 [E-pub ahead of print]. This clinical trial of 375 men randomized patients that had progressed on ADT to either Enza or bicalutamide. Median follow-up was 20 months and patients in the Enza group had a median progression free survival of 15.7 months versus a median progression-free survival of 5.8 months for those on bicalutamide.
 21. Penson DF, Armstrong AJ, Concepcion R, et al. Enzalutamide versus bicalutamide in castration-resistant prostate cancer: the STRIVE trial. *J Clin Oncol*. 2016 [E-pub ahead of print]. This clinical trial of 396 non-metastatic and metastatic CRPC patients showed that Enza is superior to bicalutamide. Men randomized to Enza treatment experienced a median PFS of 19.4 months versus those randomized to bicalutamide treatment who experienced a median PFS of 5.7 months.
 22. Li Z, Bishop AC, Alyamani M, et al. Conversion of abiraterone to D4A drives anti-tumour activity in prostate cancer. *Nature*. 2015; 523(7560):347–351. [PubMed: 26030522] This study describes the novel ⁴-3-ketosteroid metabolite of AA which is generated by 3 β -HSD type 1. This metabolite inhibits CYP17A1, 3 β -HSD type 1, 5 α -reductase and the AR and may warrant further study as a therapeutic agent.
 23. Chang KH, Li R, Kuri B, et al. A gain-of-function mutation in DHT synthesis in castration-resistant prostate cancer. *Cell*. 2013; 154(5):1074–1084. [PubMed: 23993097]
 24. Powell K, Semaan L, Conley-LaComb MK, et al. ERG/AKR1C3/AR constitutes a feed-forward loop for AR signaling in prostate cancer cells. *Clin Cancer Res*. 2015; 21(11):2569–2579. [PubMed: 25754347] This study describes the role of ERG in the regulation of expression and activity of AKR1C3. The clinical significance of this feed-forward loop is that tumors or metastatic sites that are positive for the TMPRSS2:ERG fusion may be more amenable to AKR1C3 inhibition.
 25. Carstensen JF, Tesdorpf JG, Kaufmann M, et al. Characterization of 17 beta-hydroxysteroid dehydrogenase IV. *J Endocrinol*. 1996; 150(Suppl):S3–S12. [PubMed: 8943781]

26. Bauman DR, Steckelbroeck S, Williams MV, et al. Identification of the major oxidative 3 α -hydroxysteroid dehydrogenase in human prostate that converts 5 α -androstane-3 α ,17 β -diol to 5 α -dihydrotestosterone: a potential therapeutic target for androgen-dependent disease. *Mol Endocrinol*. 2006; 20(2):444–458. [PubMed: 16179381]
27. Graff RE, Pettersson A, Lis RT, et al. The TMPRSS2:ERG fusion and response to androgen deprivation therapy for prostate cancer. *Prostate*. 2015; 75(9):897–906. [PubMed: 25728532] Results from this clinical trial and data from previous trials suggest that men with TMPRSS2:ERG positive tumors in the neoadjuvant and metastatic setting have significantly longer survival after ADT relative to men without the gene fusion.
28. Attard G, de Bono JS, Logothetis CJ, et al. Improvements in radiographic progression-free survival stratified by ERG gene status in metastatic castration-resistant prostate cancer patients treated with abiraterone acetate. *Clin Cancer Res*. 2015; 21(7):1621–1627. [PubMed: 25593303] In this clinical trial, mCRPC patients with an ERG fusion secondary to deletion of 21q22 and increased copy number of fusion sequences (class 2+ Edell) had a significantly greater improvement in radiographic progression-free survival after treatment with AA + prednisone relative to patients that had no ERG fusion.
29. Liu C, Lou W, Zhu Y, et al. Intracrine androgens and AKR1C3 activation confer resistance to enzalutamide in prostate cancer. *Cancer Res*. 2015; 75(7):1413–1422. [PubMed: 25649766] This study describes the generation of the Enza-resistant, C4-2B MDVR cell line after long-term treatment of C4-2B cells with Enza. The C4-2B MDVR cells had elevated AKR1C3 relative to the parental C4-2B cell line and inhibition of AKR1C3 re-sensitized the cells to Enza.
30. Byrns MC, Steckelbroeck S, Penning TM. An indomethacin analogue, N-(4-chlorobenzoyl)-melatonin, is a selective inhibitor of aldo-keto reductase 1C3 (type 2 3 α -HSD, type 5 17 β -HSD, and prostaglandin F synthase), a potential target for the treatment of hormone dependent and hormone independent malignancies. *Biochem Pharmacol*. 2008; 75(2):484–493. [PubMed: 17950253]
31. Klotz L, O'Callaghan C, Ding K, et al. Nadir testosterone within first year of androgen-deprivation therapy (ADT) predicts for time to castration-resistant progression: a secondary analysis of the PR-7 trial of intermittent versus continuous ADT. *J Clin Oncol*. 2015; 33(10):1151–1156. [PubMed: 25732157] This study used data from the continuous androgen deprivation arm of the PR-7 trial and found that in these men, those that achieved maximal testosterone reduction (<0.7 nmol/L in the serum) had improved cause-specific survival and duration of response to androgen deprivation.
32. Knuuttila M, Yarkin E, Kallio J. Castration induces up-regulation of intratumoral androgen biosynthesis and androgen receptor expression in an orthotopic VCaP human prostate cancer xenograft model. *Am J Pathol*. 2014; 184(8):2163–2173. [PubMed: 24949550] In this study, castration was found to up-regulate CYP17A1, AKR1C3 and HSD17B6 in a VCaP orthotopic xenograft. This platform was used to test the efficacy of Enza and ARN-509.
33. Penning TM, Steckelbroeck S, Bauman DR, et al. Aldo-keto reductase (AKR) 1C3: role in prostate disease and the development of specific inhibitors. *Mol Cell Endocrinol*. 2006; 248(1–2):182–191. [PubMed: 16417966]
34. Adeniji AO, Twenter BM, Byrns MC, et al. Development of potent and selective inhibitors of aldo-keto reductase 1C3 (type 5 17 β -hydroxysteroid dehydrogenase) based on N-phenyl-aminobenzoates and their structure-activity relationships. *J Med Chem*. 2012; 55(5):2311–2323. [PubMed: 22263837]
35. Zang T, Verma K, Chen M, et al. Screening baccharin analogs as selective inhibitors against type 5 17 β -hydroxysteroid dehydrogenase (AKR1C3). *Chem Biol Interact*. 2015; 234:339–348. [PubMed: 25555457] This study describes the evaluation of baccharin analogues for their potency and selectivity as AKR1C3 inhibitors.
36. Tamae D, Mostaghel E, Montgomery B, et al. The DHEA-sulfate depot following P450c17 inhibitions supports the case for AKR1C3 inhibition in high risk localized and advanced castration resistant prostate cancer. *Chem Biol Interact*. 2015; 234:332–338. [PubMed: 25514466] This study examines the serum androgen data from the neo-adjuvant leuprolide + AA trial and sets forth the hypothesis that the DHEA-sulfate depot may drive post-AA mCRPC and that this could be surmounted by AKR1C3 inhibition in these patients.

37. Yepuru M, Wu Z, Kulkarni A, et al. Steroidogenic enzyme AKR1C3 is a novel androgen receptor-selective coactivator that promotes prostate cancer growth. *Clin Cancer Res.* 2013; 19(20):5613–5625. [PubMed: 23995860]
38. Matsuura K, Shiraishi H, Hara A, et al. Identification of a principal mRNA species for human 3 α -hydroxysteroid dehydrogenase isoform (AKR1C3) that exhibits high prostaglandin D2 11-ketoreductase activity. *J Biochem.* 1998; 124(5):940–946. [PubMed: 9792917]
39. Fan L, Peng G, Hussain A, et al. The steroidogenic enzyme AKR1C3 regulates stability of the ubiquitin ligase Siah2 in prostate cancer cells. *J Biol Chem.* 2015; 290(34):20865–20879. [PubMed: 26160177] This study interrogated the relationship between AKR1C3 and the ubiquitin ligase Siah2 in the CWR22Rv1 prostate cancer cell line. Inhibition of Siah2 decreased AKR1C3 expression, intracellular androgen levels and growth in orthotopic prostate tumors.
40. Hagberg Thulin M, Nilsson ME, Thulin P, et al. Osteoblasts promote castration-resistant prostate cancer by altering intratumoral steroidogenesis. *Mol Cell Endocrinol.* 2015 [E-pub ahead of print]. The overexpression of steroidogenic enzymes has been characterized in bone metastatic tissue from patients. Osteoblasts were found to induce this overexpression exclusively in the osteogenic LNCaP-19 cell line but not in the osteolytic PC-3 or androgen-dependent LNCaP cell lines.

Key Points

1. 3β -HSD1 metabolizes abiraterone to D4A, its 4β -3-ketosteroid metabolite which acts as an AR antagonist.
2. The TMPRSS2:ERG gene fusion up-regulates AKR1C3 in a feed-forward manner.
3. Inhibition of AKR1C3 surmounts Enza drug resistance.
4. Intratumoral androgen metabolism plays a significant role in a subpopulation of mCRPC tumors and enzymes downstream from CYP17A1 are targets for drug development.

Figure 1.

Abiraterone is converted by 3β-HSD type 1 to its 4-3-ketosteroid metabolite, D4A. In a panel of human serum samples, D4A was found to comprise approximately 4% of total abiraterone. Characterization of the D4A metabolite has shown that it possesses inhibitory activity against CYP17A1 comparable to that of its parent compound and additionally inhibits 3β-HSD type 1, 5α-reductase and the androgen receptor (AR) [22].

Figure 2.

The ERG/AKR1C3/AR feed-forward loop plays a significant role in AR signaling in prostate cancer. A. In VCaP cells, the stable transfection of lentiviral shERG was used to knockdown ERG. As a result of shERG knockdown in the VCaP cells, concomitant reduction in AKR1C3, HSD17B6 and HSD17B4 was observed. Conversely, in LNCaP and BPH-1 cells, stable transfection of ERG-40 (the first 39 amino acids from the N-terminus are deleted) resulted in AKR1C3 over-expression. B. In the VCaP-shERG cells, reduced AKR1C3 expression also led to reduced activity as less Adione was converted to DHT relative to the VCaP-sh-scrambled control. Furthermore, reduced PSA expression was also observed in VCaP-shERG cells relative to control [24].

Figure 3.

Long-term treatment of C4-2B cells with Enza resulted in an Enza-resistant sub-line, which the authors called C4-2B MDVR. When compared to the parental C4-2B cells, the C4-2B MDVR cells were found to over-express several androgen biosynthetic enzymes including AKR1C3, CYP17A1 and HSD3B1 (the higher expressing cells are signified with a darker color). Furthermore, *in vitro* and animal xenograft studies showed that the AKR1C3 inhibitor, indomethacin re-sensitized the C4-2B MDVR cells to Enza [29].