Published in final edited form as:

J Steroid Biochem Mol Biol. 2020 February ; 196: 105499. doi:10.1016/j.jsbmb.2019.105499.

Intracrinology-Revisited and Prostate Cancer

Trevor M. Penning¹, Andrea Detlefsen²

¹Center of Excellence in Environmental Toxicology, Department of Systems Pharmacology & Translational Therapeutics

²Department of Biochemistry and Biophysics, Perelman School of Medicine, University of Pennsylvania School Philadelphia, PA

Abstract

The formation of steroid hormones in peripheral target tissues is referred to as their intracrine formation. This process occurs in hormone dependent malignancies such as prostate and breast cancer in which the disease can be either castrate resistant or occur post-menopausally, respectively. In these instances, the major precursor steroid of androgens and estrogens is dehydroepiandrosterone (DHEA) and DHES-SO₄. This article reviews the major pathways by which adrenal steroids are converted to the potent male sex hormones, testosterone (T) and 5 α -dihydrotestosterone (5 α -DHT) and the discrete enzyme isoforms involved in castration resistant prostate cancer. Previous studies have mainly utilized radiotracers to investigate these pathways but have not used prevailing concentrations of precursors found in castrate male human serum. In addition, the full power of stable-isotope dilution liquid chromatography tandem mass spectrometry has not been applied routinely. Furthermore, it is clear that adaptive responses occur in the transporters and enzyme isoforms involved in response to androgen deprivation therapy that need to be considered.

Graphical abstract

Address all correspondence to: Dr. Trevor M. Penning, 421 Curie Blvd, 1350 BRBII/IIII, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104-6084.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

abiraterone acetate; androgens; androgen receptor; aldo-keto reductase; bicalutamide; castration resistant prostate cancer; enzalutamide; hydroxysteroid dehydrogenase; leuprolide; steroid 5 α -reductase

1. Introduction

Prostate cancer is the second leading cause of cancer in men in the United States, with 174,650 new cases per year resulting in 31,620 deaths in 2019 (1). In classical work, Charles Huggins showed that patients with advanced disease could undergo remission when treated by surgical castration and adrenalectomy followed by replacement glucocorticoids (2,3). This ground breaking work not only demonstrated that the disease was androgen dependent, but that it likely relied on androgens of both testicular and non-gonadal origin. This led to androgen deprivation therapy (ADT) becoming a mainstay for advanced prostate cancer, often delivered as a combination of a LH-RH agonist (e.g. leuprolide) and an androgen receptor (AR) antagonist (bicalutamide). Early work showing that the efficacy of this combined therapy was advanced by Ferdinand Labrie (4,5). Importantly, Labrie elaborated the concept that endocrine target tissues could synthesize their own hormones in a manner that was autonomous from the control by the hypothalamic-pituitary gonadal/adrenal axis, a process subsequently termed “intracrinology” (6,7). Labrie recognized that the majority of androgens (40%) and the majority of estrogens (up to 100% postmenopausally) were synthesized in peripheral target tissues. Moreover, the genes encoding for the enzymes involved in peripheral steroidogenesis were found to be expressed in target tissues, leading to the identification of the major enzyme isoforms involved and their elevation as drug targets to treat hormone dependent malignancies.

2. Importance of Dehydroepiandrosterone (DHEA) in the Intracrine Formation of Androgens and the role of P450c17 Inhibitors

The source of non-gonadal androgens that permits prostate cancer tumors to synthesize their own potent androgens (testosterone, T; and 5 α -dihydrotestosterone, 5 α -DHT) was identified as dehydroepiandrosterone (DHEA) and its sulfonated form DHEA-SO₄ both of adrenal origin (8). DHEA-SO₄ is one of the most abundant circulating steroids in human serum (9). The conversion of C21 steroids e.g. pregnenolone to DHEA occurs in the zona reticularis of the adrenal and is catalyzed by P450c17 (*CYP17A1* here after all enzymes will be referred to by their gene names in italics) (10). This bifunctional enzyme conducts 17 α -hydroxylation to yield 17 α -hydroxypregnenolone, followed by a 17,20-lyase reaction that catalyzes cleavage of the carbon acyl-bond to yield DHEA (10). DHEA is then sulfonated by *SULT2A1* to yield DHEA-SO₄ (11). Recognition of this sequence has led to development of potent *CYP17A1* inhibitors for treatment of advanced prostate cancer, and in particular castration resistant prostate cancer (CRPC). CRPC occurs when even in the presence of chemical or surgical castration, the tumor reappears and remains androgen dependent as signaled by rising levels of prostate serum antigen (PSA). CRPC is the lethal form of prostate cancer and accounts for 30,000 deaths in the US annually.

Ketoconazole was one of the first *CYP17A1* inhibitors (12,13) used and has since been replaced by abiraterone acetate (14,15) and second generation *CYP17A1* inhibitors galeterone (16). In intact males, abiraterone acetate blocks Leydig cell steroidogenesis, DHEA formation in the adrenal gland, and possibly tumor tissue steroidogenesis as well. *CYP17A1* inhibitors are thus the equivalent of a chemical castration and adrenalectomy. In castrated males, abiraterone acetate blocks adrenal and tumor steroidogenesis only. Abiraterone acetate must be given with prednisone to block the production of adrenocorticotrophic hormone (ACTH) that occurs in the absence of cortisol biosynthesis to prevent the build-up of deoxycorticosterone in the adrenal and prevent mineralocorticoid excess (17). Abiraterone acetate has been approved by the FDA and is effective in increasing progression free survival in CRPC, prolonging life by 3–17 months before drug resistance emerges. These findings beg the question as to the pathway(s) that are responsible for the intracrine formation of androgens in normal prostate and prostate cancer, the enzyme isoforms involved, how they may be regulated by androgen deprivation, and how they may contribute to drug resistance.

3. Pathways to Testosterone to 5 α -DHT in the Prostate

DHEA-SO₄ enters prostate cancer cells via an organic anion transporter protein (OATP of the solute organic anion transporters gene family *SLCO*). Steroid sulfatase (*STS*) then cleaves the sulfate group to yield free DHEA. At this point there are four pathways that can lead to T and 5 α -DHT.

In the canonical (classical pathway), DHEA is converted to 4-androstene-3,17-dione (⁴-AD) by the bifunctional 3 β -hydroxysteroid dehydrogenase (HSD)/3-ketosteroid isomerase (*HSD3B1*). ⁴-AD is then reduced by 17 β -HSD isoforms to yield T which is reduced to 5 α -DHT by 5 α -reductase isoforms (*SRD5A*) (18). In the alternate pathway, ⁴-AD is converted to 5 α -androstane-3,17-dione (5 α -Adione) by *SRD5A* isoforms and 17 β -HSD reduces 5 α -Adione to 5 α -DHT. This pathway by-passes T production altogether and is referred to as the “alternate or 5 α -Adione” pathway (19). In the third pathway, DHEA is reduced to 5-androstene-3 β ,17 β -diol (5-Adiol) by 17 β -HSD isoforms. The 5-Adiol is then converted to testosterone by *HSD3B1*.

A fourth pathway, known as the “backdoor pathway”, involves conversion of progesterone to 5 α -dihydroprogesterone which is further reduced to produce allopregnanolone (20). Allopregnanolone is then converted to androsterone by *CYP17A1*. The androsterone product is subsequently reduced to 5 α -androstane-3 α ,17 β -diol (3 α -Adiol) by a reductive 17 β -HSD which is then converted to DHT by an oxidative 17 β -HSD. This pathway is known as the “backdoor pathway” since the precursor steroid is 3 α -Adiol and T is not involved, Figure 1.

A fifth pathway involving 11-oxygenated steroids of adrenal origin has also garnered attention. 11 β -hydroxy-4-androstene-3,17dione, which is a major androgen found in the adrenal vein (21), can be converted into 11-oxo-4-androstene-3,17dione in prostate cancer cells and tissue. 11-oxo-4-androstene-3,17dione can then be converted to both 11-oxo-testosterone and 11-oxo-5 α -DHT using the same enzymes that convert 4-androstene-3,17-dione to T and 5 α -DHT (22). Interest exists in this pathway since 11-oxo-testosterone and

11-oxo-5 α -DHT have the same potency as T and 5 α -DHT in androgen receptor reporter gene assays, but they are rarely measured in patient samples even though high levels would provide a mechanism of castration resistance (23).

Labrie's group was a leader in identifying and characterizing the discrete HSD isoforms involved in these steroid transformations in target tissues with a focus on *HSD3B* and 17 β -HSD isoforms. In the former case his group characterized the human genes (*HSD3B1* and *HSD3B2*) and their tissue specific expression (24–28). In the latter case his group helped to elucidate the key role of human 17 β -HSD isoforms in steroidogenesis in target tissues (29,30). It was found that the reductive 17 β -HSDs convert the less potent 17-oxo-derivatives to the more potent 17 β -hydroxy derivatives while the oxidative 17 β -HSDs convert the more potent 17 β -hydroxy derivatives to the less potent 17-oxo-derivatives. Thus, the discrete 17 β -HSD isoforms can act as molecular switches to regulate the amount of ligand available for the androgen or estrogen receptor; a process known as the pre-receptor regulation of hormone action. The 17 β -HSD isoforms are often referred to as belonging to the *HSD17B* family, however, the 14 human isoforms belong to two different gene/protein super-families, the short-chain dehydrogenase/reductases (SDRs) and the aldo-keto reductases (AKRs), where 17 β -HSD type 5 α is *AKR1C3* (31), Table 1.

3. 1. Interrogation of Pathways to T and 5 α -DHT.

Work aimed at identifying the major pathways to T and 5 α -DHT has been conducted using prostate tissue from normal and diseased patients, prostate cancer cell lines, and through ex vivo experiments in xenograft models. Since both normal and diseased patient tissue are used it is likely incorrect to assume that biosynthetic pathways to T and DHT are always the same. This could also be true of prostate cancer cell lines, which may have undergone different adaptations in response to therapy. Importantly, many of the prostate cancer cell lines are from patients in which the AR was mutated to become promiscuous in their ligand specificity, and in a similar way these cell lines may have adapted to produce promiscuous ligands. Moreover, concentrations of precursor steroids used in metabolism experiments may not reflect human serum concentrations. For example, the number of studies using DHEA, DHEA-SO₄ or ⁴-AD as precursors at the prevailing concentrations present in the serum of castrate men are few, and the same can be said of C21 precursors of androgens, Table 2. This is in part due to the dependence on radiotracers that are of limited availability. In addition, many studies using radiotracers have relied on radiochromatography and characterization of products by retention time versus an authentic synthetic standard. A key feature of the chromatographic method is to ensure that isomeric steroids are well separated. For example, DHEA, testosterone and epi-testosterone are all isomers. Similarly, DHT, androsterone and epiandrosterone are all isomers. By contrast, the gold standard for measuring physiologically relevant concentrations of androgens and their precursors would be stable-isotope dilution liquid chromatography tandem mass spectrometry. This highly sensitive method permits any androgen precursor to be used at often very low physiologically relevant concentrations, while the appropriate isotopically labeled internal standard serves to correct for any losses in the analytical method. Furthermore, the identities of androgen products are pinpointed by multiple readouts including retention time, correct molecular ion, and ion transitions in the

MS/MS mode. The use of state-of-the art liquid chromatography tandem mass spectrometry to support analyte identity in metabolism studies is now only beginning to be applied.

In other approaches, qRT-PCR showing expression of steroidogenic enzymes at the RNA level have been prematurely interpreted as an indicator that pathways involving these enzymes are intact, without performing necessary flux measurements. With these caveats some key papers will be reviewed.

3. 1. 1. De novo androgen biosynthesis.—Prostate cancer cell lines have been shown to convert [^{14}C]-acetate into C21 and C19 steroids in LNCaP xenografts grown in a castrate murine model. Ex vivo [^{14}C]-acetate was converted to progesterone, 17 α -hydroxyprogesterone and 5 α -DHT with 4%, 1.5% and 8.3% conversion respectively, but no T formation was observed. These studies relied on HPLC with radiometric detection of the analytes (34). Androgen starved LNCaP cells were also found to convert [^3H]-progesterone to 17 α -hydroxyprogesterone, 5 α -DHP, androsterone, and DHT, where analytes were matched by retention time using LC-MS. In these experiments only modest amounts of C21 steroids were converted to C19 steroids. It was concluded that the modest appearance of C19 steroids was likely due to the low 17,20-lyase activity of *CYP17A1* (35). Furthermore, the detection of the pregnanes was interpreted to support the involvement of the backdoor pathway to 5 α -DHT. However, the modest conversion of acetate into pregnanes and their subsequent conversion to androgens places the importance of *de novo* synthesis in doubt since a large reservoir of DHEA-SO₄ remains even in the presence of maximal inhibition of *CYP17A1* by abiraterone acetate, Table 3 (33,36).

3.1.2. The Canonical Pathway.—The presence of the canonical pathway was supported by studies on freshly harvested prostate tissue from benign, hormone-naïve, and hormone-refractory prostate cancer. These tissues were incubated in the presence of 5 μM cholesterol, progesterone, DHEA, androstenedione, or testosterone for 96 hours, after which concentrations of 15 different steroids in the conditioned media were measured by gas chromatography–mass spectroscopy. Changes in the expression of androgen synthetic and/or degradative enzymes were also determined by expression microarray and qPCR and were correlated with the metabolic profiles. Only incubation with $^4\text{-AD}$ gave rise to significant concentrations of testosterone. Although this was observed in all tissue types, it occurred to a significantly greater degree in hormone-refractory tissue than in hormone-naïve prostate cancer tissue. Gene set enrichment analysis of expression microarray data revealed significant upregulation of *HSD17B* expression including AKR1C3. Importantly, no evidence was found to support contributions from either the “backdoor” or “the alternative or “5 α Adione” pathways (18). Notably, this study used supraphysiologic levels of $^4\text{-AD}$. The study was also criticized by Auchus since only one end point measurement was used to interrogate the different pathways to androgens, making it impossible to identify product-precursor relationships, and no attempt was made to measure 5 α -Adione (37).

3. 1. 3. Alternative Pathway.—Support for the alternative pathway that by-passes T altogether was detailed in six CRPC cell lines (LNCaP, VCaP, LAPC4, 22Rv1, DU145, C4–2) by showing that they preferentially converted $^4\text{-AD}$ to 5 α -Adione and not T. Prostate tissue from metastatic sites and two CRPC patients showed similar findings. The conversion

of 4α -AD to 5α -Adione in CRPC was also found to involve 5α -reductase type 1 (*SRD5A1*) and not 5α -reductase type 2 (*SRD5A2*). Genetic knockdown of *SRD5A1* prevented the formation of 5α -Adione in the cell lines, and xenograft studies using these cells showed an attenuation of tumor formation that could be surmounted by the administration of 5α -Adione (19). Unfortunately, the outcome of these studies could have been biased as they were performed with supraphysiologic concentrations of 4α -AD (100 nM) when the prevailing serum concentration is 2.5 nM.

Seventeen patients with clinically localized prostate cancer were consented to obtain fresh tissues after radical prostatectomy (38). Prostate tissues were cultured *ex vivo* in media spiked with 1 μ M each of 4α -AD and T, and stable isotopic tracing detected by LC-MS was employed to simultaneously follow the enzymatic conversion of both these precursor steroids into 5α -DHT. It was found that both steroids were substrates for *SRD5A* and were metabolized equally in primary prostate tissues. However, in CRPC, the conversion of 4α -AD to 5α -DHT was favored over the conversion of T to 5α -DHT further supporting the bypass mechanism. Unfortunately, the concentrations of 4α -AD and T were much higher than the prevailing serum concentrations in humans. Moreover, no studies have been reported in which prevailing serum concentrations of DHEA-SO₄ or DHEA were substituted as precursors. Thus, interrogation of the sequence DHEA-SO₄ to DHEA to 5-androstenediol to T has been unaddressed. Thus, the main pathway by which DHEA-SO₄ and DHEA are converted to T and DHT remains unclear, and is a controversy that is complicated by the suggestion that no single pathway may be conserved in all CRPC patients (39).

3. 1. 4. Backdoor Pathway.—The backdoor pathway was interrogated in LNCaP and 22Rv1 prostate cancer cells and human prostate tissues following incubation with steroid precursors (22-OH cholesterol, pregnenolone, 17α -hydroxypregnenolone, progesterone and 17α -hydroxyprogesterone). Increases in the production of 5α -dihydroprogesterone, 5α -pregnane-3,17-diol-20-one and allopregnanolone were observed in cells and tissues. However, the conversion of C21 to C19 steroids was not observed, suggesting the lack of functional 17,20-lyase activity in *CYP17A1* (40).

3. 1. 5. 11-Oxygenated Androgen Pathway.—This pathway was investigated using normal prostate epithelial cells (PNT2) and the androgen dependent prostate cancer cell line (LNCaP). UPC² MS/MS was used to monitor the metabolism of 4-androstene-3,17-dione and 11β -hydroxy-4-androstene-3,17-dione. In PNT2 cells, 60% of 4-androstene-3,17-dione was metabolized to 40% 5α -Adione, 10% T and 10% androsterone. Only 20% of 11β -hydroxy-4-androstene-3,17-dione was metabolized to 11-oxo-4-androstene-3,17-dione, 11-oxo- 5α -DHT, and 1103B2;hydroxy- 5α -Adione. By contrast in LNCaP cells, 4-androstene-3,17-dione (90%) was metabolized predominately to androsterone-3 β -glucuronide, while 11β -hydroxy-4-androstene-3,17-dione (80%) was metabolized to 11-oxo-4-androstene-3,17-dione and 11-oxo-T, demonstrating the importance of 11β -hydroxysteroid dehydrogenase type 2 in this pathway (22).

3. 1. 6. Deficiencies in Glucuronidation.—Glucuronidation of hydroxyandrogens is a major pathway to eliminate potent androgens in human prostate. Deficiencies in this pathway can lead to elevated intratumoral androgens and contribute to resistance to

androgen deprivation therapy. LNCaP and C4-2 cell lines normally have intact glucuronidation pathways that rapidly conjugate and inactivate testosterone and 5 α -DHT to limit androgen signaling. However, in a castrate environment these cells may become glucuronidation deficient and resistant to androgen deprivation. Using CRISPR/Cas9-mediated gene ablation, loss of UDP glucuronosyltransferase family 2 member B15 α (UGT2B15) and UGT2B17 was sufficient to restore free 5 α -DHT, sustain androgen signaling, and lead to the development of castration resistance (41). However paradoxically, several studies show that these enzymes are elevated in CRPC and may represent adaptive changes to make the tumor independent of AR ligands (42–44).

4. Contribution of Individual Enzymes & Polymorphisms.

Following castration, DHEA-SO₄ is likely the main serum precursor of T and 5 α -DHT in the prostate. Significant effort has been made to identify the discrete enzyme isoforms involved in the intracrine formation of these hormones, identify polymorphic variants and elucidate mechanisms of gene regulation.

4.1. Organic Anion Transporters.

DHEA-SO₄ is transported into the prostate by *SLCO1A2*, *SLCO1B1*, *SLCO1B3* and *SLCO2B1* (45,46). *SLCO* transcripts were markedly upregulated in LNCaP and 22Rv1 cells upon growth in androgen depleted media to mimic ADT. *SLCO1A2*, *SLCOB1* and *SLCOB2* mRNA was increased 4-fold, 2.5 fold and 1.74 fold, respectively. This increased expression was accompanied by increased cellular uptake of [³H]-DHEA-SO₄ and cellular proliferation stimulated by DHEA-SO₄. Cellular proliferation in response to DHEA-SO₄ was attenuated by *SLCO1A2* knockdown implicating its involvement. *SLCO1A2* was downregulated by 5 α -DHT suggesting that its expression is repressed by androgens (45,46).

SLCO1B1- and *SLCO2B1*-expressing prostate cancer xenografts also showed a 3.9-fold and 1.9-fold increase in DHEA-SO₄ uptake, respectively (47). These genes were overexpressed in CRPC metastases and were associated with an increase in prostate cancer mortality (48). Importantly, SNPs in these transporters can affect patient outcome. Three SNPs in *SLCO2B1* rs1242149, [935G>A; Arg312Gln], rs1789693 and rs1077858 were associated with decreased time to progression in patients on ADT ($p < 0.05$). These findings indicate that changes in *SLCO* expression and genotype can determine response to ADT.

4.2. Steroid Sulfatase.

There is only one steroid sulfatase (STS) gene responsible for the liberation of free DHEA from DHEA-SO₄. However, STS is regulated by six different tissue specific promoters. STS has been detected in prostate tissue where the highest activity is in the prostatic epithelium (49,50); and it is also expressed in LNCaP cells (51,52).

4.3. 3 β -Hydroxysteroid dehydrogenase/ketosteroid isomerase (3B-HSD/KSI).

3 β -HSD/KSI (*HSD3B*) catalyzes the conversion of DHEA to ⁴-AD. There are two isoforms of this bifunctional enzyme: *HSD3B1* and *HSD3B2*. *HSD3B1* is expressed in peripheral tissues including prostate, breast, skin and placenta. By contrast *HSD3B2* is

expressed in the adrenal glands and gonads. A major germline single nucleotide polymorphism exists in the *HSD3B1* gene which involves an A→C missense mutation at position 1245 and causes a N → T change in the protein sequence. This change causes a loss in protein ubiquitination and proteasomal degradation, and an increase in enzyme stability leading to enhanced conversion of DHEA to ⁴-AD and ultimately formation of T and 5α-DHT (53,54). This mutation is selected following ADT and may contribute to drug resistance in CRPC. Conversion of DHEA to downstream metabolites was measured in LNCaP cells which express the mutant allele. Under these conditions 90% [³H]-DHEA was consumed and 40%, 8% and 9% of the label was found in ⁴-AD, 5α-Adione and 5α-DHT, respectively. This conversion was blocked by sh-RNA for the mutant allele. It is noteworthy that a large portion of the DHEA precursor and ⁴-AD were unaccounted for in this study.

Because abiraterone is a ⁵-ene with a 3β-hydroxy group it is not surprising that *HSD3B1* converted abiraterone to the ⁴-3-ketosteroid metabolite (⁴-Abi). *In vitro*, ⁴-Abi was comparable in potency to abiraterone as a *CYP17A1* inhibitor and additionally acted as an AR antagonist (55,56). Formation of ⁴-Abi was originally proposed as an alternative mechanism of action of abiraterone acetate, but since ⁴-Abi is subsequently reduced by 5α-reductase to 5α-Abi which is an AR agonist, (54) there is a need to inhibit its formation by *HSD3B1*. Abiraterone acetate was also found to inhibit *HSD3B1*, thus higher serum concentrations of abiraterone acetate could block androgen synthesis catalyzed by the more stable variant (57). These findings suggest that it would be advantageous to combine 5α-reductase inhibitors with abiraterone for the treatment of CRPC. In a clinical trial, abiraterone acetate plus prednisone was administered for two 4 week cycles after which a high dose of dutasteride was administered. Following the addition of dutasteride there were no further changes in serum 5α-DHT and no further decreases in PSA levels, suggesting that sequential administration of these drugs had no benefit (58).

4.4. 17β-Hydroxysteroid dehydrogenases (HSD17B and AKR1C3).

There are a number of reductive human *HSD17B* genes which could be involved in formation of potent 17β-hydroxyandrogens T and 5α-DHT from their inactive precursors, ⁴-AD and 5α-Adione, respectively (59). Of these, *HSD17B1* is considered the estrogenic enzyme that converts estrone to 17β-estradiol in peripheral tissues (29,60,61). *HSD17B3* is the androgenic enzyme which converts ⁴-AD to T and is expressed predominately in the Leydig cells in the testis (62). This leaves AKR1C3 (17β-HSD type 5) as the available 17-ketosteroid reductase in the prostate for the synthesis of these potent androgens, see Table 1.

AKR1C3 was originally cloned from a prostate cDNA library (63). The *AKR1C3* gene gives rise to two potential splice variants: P42330–1, which lacks amino acids 1–119, and P42330–2, which contains only the first 204 amino acids. Evidence that these transcripts are translated into proteins is lacking and neither are predicted to be catalytically active.

There is overwhelming evidence that AKR1C3 is overexpressed in CRPC as part of an adaptive response to androgen deprivation therapy (ADT). This is seen in prostate cancer cells maintained in androgen depleted media (64), in xenograft models of CRPC (42,65), in patients with localized advanced prostate cancer, and in soft-tissue metastasis (43,66).

AKR1C3 is one of the most highly upregulated steroidogenic genes in CRPC as measured by Affymetrix microarray, qRT-PCR and by immunohistochemistry (IHC), Fig. 3 (43).

Nine clinical studies have reported similar findings for AKR1C3 expression in biospecimens from CRPC patients (18,42,64–66,68–70). In four studies there was a significant correlation between transcript levels and AKR1C3 IHC staining, where $n = 11, 51, 41,$ and 40 patients, respectively (65,68,70). Approximately one-third of metastatic CRPC (mCRPC) patients overexpress AKR1C3, but these estimates are based on specimens from patients whose prior exposure to second line ADT therapy was not reported. If CRPC patients were stratified based on prior second-line ADT, then the percentage of patients overexpressing AKR1C3 is anticipated to be even higher.

Data from Gene Expression Omnibus for 25 mCRPC tumors revealed a significant correlation ($p < 0.0001$ $r = 0.69$) between AKR1C3 and ERG co-expression. The TMPRSS2-ERG fusion protein overrides the repressive activity of AR on the AKR1C3 promoter to establish a positive feed-back loop whereby AKR1C3 synthesizes potent androgens to induce its own transcription factor in late stage disease (69). Using the Oncomine database to construct Kaplan-Meier survival curves for 363 prostate cancer patients it was found that *AKR1C3* expressors and low *TMPRSS2-ERG* expressors had a median survival of 12–13 years, but patients with tumors that were high *AKR1C3* and *TMPRSS2-ERG* expressors had a reduced median survival of 7–10 years (69). Accumulating evidence suggests that AKR1C3 plays an important role in drug resistance to ENZ and AA. For example, ENZ and AA resistant C4–2B cells overexpress AKR1C3 and their growth in xenograft models was blocked by indomethacin, an AKR1C3 competitive inhibitor (71,72).

AKR1C3 also contains a number of non-synonymous SNPs in the coding region with minor allelic frequencies (MAF) of greater than 0.1%: of H5Q (0.42 MAF), R66Q (0.023), R76G (0.004); E77G (0.037 MAF), K104D (0.158), P180S (0.086 MAF), K183R (0.0026); and AKR1C3 R258C (0.033). Several of these variants all decreased the 17-keto reduction of exemestane by 20–40 fold but their effect on the conversion of 4-androstene-3,17-dione to T or 5 α -Adione to DHT has yet to be determined (73). Mapping of these variants to the AKR1C3 crystal structure shows that they do not reside in the cofactor or steroid binding cavity, see Fig. 4, but could affect protein stability.

There are also a number of oxidative *HSD17B* isoforms that can convert potent 17 β -hydroxysteroids back to their inactive 17-keto forms, these include *HSD17B2* and *HSD17B4*, Table 1. In the former case, *HSD17B2* expression was reduced as prostate cancer progressed. In contrast, *HSD17B2* overexpression suppressed androgen-induced cell proliferation and growth of prostate cancer xenografts. Multiple mechanisms contribute to loss of *HSD17B2* expression in prostate cancer including DNA methylation and mRNA alternative splicing (74). Sharifi and coworkers found that 5 α splice variants exist for the oxidative *HSD17B4* and, unexpectedly, expression is increased in CRPC. However, only variant 2 had functional activity and could oxidize T to 4-AD and 5 α -DHT to 5 α -Adione and was suppressed in CRPC. Thus, *HSD17B2* and *HSD17B4* repression may also contribute to CRPC progression (75).

4. 5. Regulation of AR ligand occupancy.

5 α -DHT is the most potent natural ligand for the AR. The amount of ligand available can be determined by its reduction to 3 α -androstane-3 α , 17 β -diol and 3 β -androstane-3 β , 17 β -diol, and the oxidation of these diols back to 5 α -DHT. The enzyme implicated in the conversion of 5 α -DHT to 3 α -androstane-3 α , 17 β -diol based on kinetic constants, product profiling, and expression in prostate epithelial and stromal cells is *AKR1C2* (type 3 3 α -HSD) (76,77). The *AKR1C2* gene gives rise to three splice variants. Two of these variants (*AKR1C2-001*; CCDS7062 and *AKR1C2-201*; CCDS7062) differ in the length of their 5'-UTR but give rise to the full length AKR1C2 protein of 323 amino acids. The third variant (*AKR1C2-203*; CCDS44350) has lost 5-exons and would form an inactive 139 amino acid protein. The NCBI data base lists four nsSNPs in *AKR1C2* (T231; P119T; K185E; and R258C) in locations of evolutionary conserved amino acids, where R258C has a MAF of 0.064. The effect of allelic variation in *AKR1C2* on the *in vitro* metabolism of 5 α -DHT has been examined. Unfortunately, the authors examined the effect of nsSNP variants following expression in Sf9 insect cell lysates and used a catalytic inactive mutant Y55F as a control. Under these conditions a significant background turnover of 5 α -DHT was noted in the presence of the Y55F mutant making it difficult to interpret these data. It was found that F46Y (0.0649 MAF) and L172Q (not in NCBI) reduced the apparent $V_{\max}$ and that L172Q, K185E, and R258C all reduced the apparent K_m . However, their effect on the utilization ratio $V_{\max}/K_m$ for reduction of DHT by these variants was modest and varied by only 2–3 fold (78). Using an approach similar to that which implicated AKR1C2 in the conversion of DHT to 3 α -androstane-3 α , 17 β -diol, AKR1C1 was implicated in the conversion of DHT to 3 α -androstane-3 α , 17 β -diol (76,79).

A systematic study of all the potential SDR enzymes that could convert 3 α -androstane-3 α , 17 β -diol to DHT by the backdoor pathway was performed by transfection studies. Candidate enzymes involved were 11-*cis* retinol dehydrogenase (RODH 5), L-3-hydroxyacyl coenzyme A dehydrogenase, RODH like 3 α -HSD (RL-HSD), novel type of human microsomal 3 α -HSD, and retinol dehydrogenase 4 (*RODH4*). Of the enzymes that oxidized 3 α -diol back to 5 α -DHT *RODH5*, *RODH4*, and RL-HSD (*HSD17B6*) were the most efficient. Steady-state kinetic parameters indicated that *RODH4* and *RL-HSD* were high-affinity, low-capacity enzymes whereas *RODH5* was a low-affinity, high-capacity enzyme. AR-dependent reporter gene assays showed that *RL-HSD*, *RODH5*, and *RODH4* shifted the dose-response curve for 3 α -diol by 100-fold, yielding EC₅₀ values of 2.5×10^{-9} M, 1.5×10^{-9} M, and 1.0×10^{-9} M, respectively, when compared with the empty vector which gave an EC₅₀ value of 1.9×10^{-7} M. qRT-PCR indicated that L-3-hydroxyacyl coenzyme A dehydrogenase and *RL-HSD* were expressed more than 15-fold higher compared with the other candidate oxidative enzymes in human prostate and that RL-HSD and AR were colocalized in primary prostate stromal cells. These data showed that the major oxidative 3 α -HSD in normal human prostate is *RL-HSD* (*HSD17B6*) and may be a new therapeutic target for treating prostate diseases (80). This enzyme was subsequently shown to be the important enzyme for the conversion of 3 α -diol to 5 α -DHT in prostate cancer cell lines and in xenograft models by Mohler and colleagues (81,82).

5. Enzyme Inhibitors

Labrie maintained that by identifying the discrete enzyme isoforms responsible for the intracrine formation of steroid hormones, targets for drug development would be revealed. In prostate cancer, a case could be made to inhibit *STS*, *HSD3B1*, *HSD17B6* and *AKR1C3*.

5. 1. Steroid Sulfatase Inhibitors.

The steroid sulfatase inhibitor irosustat (STX-64) inhibited DHEA-SO₄ mediated growth of prostate cancer cells *in vitro* (45). The drug was advanced to a phase I clinical trial in CRPC patients where it was found to be well tolerated and inhibited the conversion of DHEA-SO₄ to DHEA (83).

5. 2. HSD3B1 Inhibitors.

The similarity in amino-acid sequences for *HSD3B1* and *HSD3B2* suggests that it would be challenging to develop inhibitors that would not block steroidogenesis in gonad, adrenal and peripheral tissues simultaneously. Trilostane and epostane are two compounds that show some discrimination between isoforms, but the difference in IC₅₀ values is unimpressive (84–86).

5. 3. HSD17B6 Inhibitors.

Compounds that specifically inhibit *HSD17B6* would block the backdoor pathway to 5 α -DHT. However since there are multiple pathways to 5 α -DHT, it is likely that inhibition of this single pathway could lead to adaptive upregulation of one or of the others to synthesize compensatory amounts of 5 α -DHT. With this caveat, combined inhibition of 5 α -reductase and *HSD17B6* has been described as a therapeutic strategy (87).

5.4. AKR1C3 Inhibitors.

AKR1C3 acts downstream from *CYP17A1* and plays a pivotal role in all pathways to 5 α -DHT. Moreover, it is involved in metabolism of 11-oxo-⁴-AD to 11-oxo-T and conversion of 11-oxo-5 α -Adione to 11-oxo-DHT (88). Thus, adaptive responses in the canonical, alternative and backdoor pathways due to ADT would be effectively surmounted by inhibition of *AKR1C3*. The challenge is to develop *AKR1C3* isoform selective inhibitors that do not inhibit the highly related *AKR1C1* and *AKR1C2* enzymes. Following the observation that *AKR1C* enzymes demonstrate variable sensitivity to inhibition by NSAIDs (89,90), NSAID analogs have been repurposed to generate compounds that inhibit only *AKR1C3* and not *AKR1C1*, *AKR1C2* or *AKR1C4* and lack inhibition of PGH₂ synthases (COX-1 and COX-2). This has led to a series of drugs including indomethacin analogs (91), *N*-phenylaminobenzoates (92,93), and *R*-naproxen analogs (94) that have the desired properties. Moreover, indomethacin was found to surmount abiraterone and enzalutamide drug resistance in xenograft models over expressing *AKR1C3* (71,72). These observations lead to the development of ASP9521 by Astellas. ASP9521 has attractive preclinical data and was subsequently taken into a phase1/1b clinical trial in CRPC (95,96). The primary end-point was progression free survival and a secondary end point was a reduction in PSA. The drug was found to be well tolerated but without efficacy. However, tumor biopsies were not pre-screened for the expression of *AKR1C3* and patients were excluded if they had been

on prior ADT, the very treatment that would upregulate *AKR1C3* expression in the first place. Thus the trial may have failed due to inadequate patient selection.

Two other drug candidates which act as inhibitors of AKR1C3 are of interest, these are GTx-560 and BMT4-158. GTx-560, a competitive inhibitor of AKR1C3, also blocked a hitherto unknown property of the enzyme which was its AR coactivator function (97). BMT4-158, also a competitive inhibitor of AKR1C3 was found to also act as a AR antagonist by competing for the binding of R1881 (98). Since combination therapy may override adaptive responses that may compensate for AR antagonism or inhibition of prostate androgen biosynthesis, bifunctional compounds provide important leads for CRPC treatment.

5.5. Steroid 5 α -Reductase Inhibitors (SRD5A).

There are two SRD5A isoforms in human prostate and the isoform most implicated in the conversion of T to 5 α -DHT is *SRD5A2*. However, in the 5 α -Adione pathway, evidence suggests that SRD5A1 is the important driver of tumor growth. Thus, a cautious approach would be the use of a dual SRD5A1 and SRD5A2 inhibitor, e.g. dutasteride. However even with inhibition of this enzyme, T would continue to build up and may be sufficient to cause tumor growth (99). In fact data from the Montgomery group showed that in advanced mCRPC, the tumor may be more dependent on T than DHT based on measurement of their ratios in biopsy material (100). Currently, there is a FDA black-box warning on the use of *SRD5A* inhibitors for the treatment of prostate cancer, since chemopreventive clinical trials indicated that both finasteride (in the Prostate Cancer Prevention Trial) and dutasteride (REDUCE Trial) could give rise to more advanced tumors (101).

6. Conclusions.

Fredinand Labrie was the father of the field of “intracrinology”. His legacy solidified the concept that hormone dependent malignancies synthesize their own steroid hormones and that the major precursor for this synthesis in post-menopausal women and castrate or aging men was DHEA. While there is much debate as to whether DHEA is itself a hormone, Labrie was correct. Many steroid endocrinologists have embraced his concept, and his group and others have devoted their efforts to identify the discrete enzymes involved in this process with the intent of developing inhibitors for the treatment of hormone dependent malignancies. In the case of prostate cancer, competing pathways exist to form the potent ligands T and 5 α -DHT. It is likely that no single pathway will dominate and their involvement in drug resistance may be dependent on the tumor itself, supporting precision approaches to the treatment of disease. This task is made even more daunting because as one pathway is inhibited another may compensate. In addition, prostate cancer that is independent of AR ligand may emerge due to the formation of AR-splice variants and substitution of other nuclear receptors for the AR, e.g. the GR (102).

Funding sources:

This work was supported by a grant from the National Institute of Environmental Health Sciences P30-ES013508 awarded to TMP.

Abbreviations:

ADT	androgen deprivation therapy
AKR	aldo-keto reductase
AR	androgen receptor
DHEA	dehydroepiandrosterone
5α-DHT	5 α -dihydrotestosterone
HSD	hydroxysteroid dehydrogenase
KSI	ketosteroid isomerase
LH	luteinizing hormone
OATP	organic anion transporter protein

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics. *CA Cancer J Clin* 2019; 69:7–34. DOI: 10.3322/caac.21551. [PubMed: 30620402]
2. Huggins CB, and Hodges CV. Studies on prostatic cancer 1. Effect of castration, estrogen and androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res* 1941; 1:293–397. DOI: 10.3322/canjclin.22.4.232.
3. Huggins CB. Two principles in endocrine therapy of cancers: Hormone deprivation and hormone interference. *Cancer Res* 1965; 25:1163–1167 [PubMed: 5897831]
4. Labrie F, Dupont A, Belanger A, Cusan L, Lacourciere Y, Monfette G, Laberge JG, Emond JP, Fazekas AT, Raynaud JP, Husson JM. New hormonal therapy in prostatic carcinoma: combined treatment with an LHRH agonist and an antiandrogen. *Clin Invest Med* 1982; 5:267–275. DOI: 10.1016/0022-4731(83)90046-8. [PubMed: 6819101]
5. Labrie F, Dupont A, Belanger A, Emond J, Monfette G. Simultaneous administration of pure antiandrogens, a combination necessary for the use of luteinizing hormone-releasing hormone agonists in the treatment of prostate cancer. *Proc Natl Acad Sci U S A* 1984; 81:3861–3863. DOI: 10.1073/pnas.81.12.3861. [PubMed: 6427777]
6. Labrie F. Intracrinology. *Mol Cell Endocrinol* 1991; 78:C113–118. DOI: 10.1016/0303-7207(91)90116-a. [PubMed: 1838082]
7. Labrie F, Belanger A, Simard J, Luu-The V, Labrie C. "Intracrinology". Autonomy and freedom of peripheral tissues]. *Ann Endocrinol (Paris)* 1995; 56:23–29 [PubMed: 7747916]
8. Labrie F, Belanger A, Simard J, Van L-T, Labrie C. DHEA and peripheral androgen and estrogen formation: intracrinology. *Ann N Y Acad Sci* 1995; 774:16–28. DOI: 10.1111/j.1749-6632.1995.tb17369.x. [PubMed: 8597456]
9. Freidrich N, Volze H, Roskopf D, Steveling A, Krebs A, Nauck M, Wallaschofski H. Reference ranges for serum dehydroepiandrosterone sulfate and testosterone in adult man. *J Andrology* 2008; 29:610–617. DOI: 10.2164/jandrol.108.005561.
10. Chung BC, Picado-Leonard J, Haniu M., Bienkowski M., Hall PF, Shively JE, Miller WL. Cytochrome P450c17 (steroid 17 α -hydroxylase/17,20 lyase): cloning of human adrenal and testis cDNAs indicates the same gene is expressed in both tissues. *Proc Natl Acad Sci U S A* 1987; 84:407–411. DOI: 10.1073/pnas.84.2.407. [PubMed: 3025870]
11. Mueller JW, Idkowiak J, Gesteira TF, Vallet C, Hardman R, van den Boom J, Dhir V, Knauer SK, Rosta E, Arlt W. Human DHEA sulfation requires direct interaction between PAPS synthase 2 and DHEA sulfotransferase SULT2A1. *J Biol Chem* 2018; 293:9724–9735. DOI: 10.1074/jbc.RA118.002248. [PubMed: 29743239]

12. Couch RM, Muller J, Perry YS, Winter JS Kinetic analysis of inhibition of human adrenal steroidogenesis by ketoconazole. *J Clin Endocrinol Metab* 1987; 65:551–554. DOI: 10.1210/jcem-65-3-551. [PubMed: 3497939]
13. Ayub M, Levell MJ Inhibition of testicular 17 alpha-hydroxylase and 17,20-lyase but not 3 beta-hydroxysteroid dehydrogenase-isomerase or 17 beta-hydroxysteroid oxidoreductase by ketoconazole and other imidazole drugs. *J Steroid Biochem* 1987; 28:521–531. DOI: 10.1016/0022-4731(87)90511-5. [PubMed: 2824931]
14. Attard G, Reid AHM, Yap TA, Raynaud F, Dowsett M, Settatree S, Barrett M, Parker C, Martinins V, Folkard E, Clark J, Cooper CS, Kaye SB, Dearnaley D, Lee G, and de Bono JS Phase 1 clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. *J Clin Oncol* 2008; 28:4563–4571. DOI: 10.1200/JCO.2007.15.9749.
15. Attard G, Reid AH, Olmos D, and de Bono JS Antitumor activity with CYP17 blockade indicates that castration-resistant prostate cancer frequently remains hormone driven. *Cancer Res* 2009 69:4937–4940. DOI: 10.1158/0008-5472.CAN-08-4531. [PubMed: 19509232]
16. Njar VC, Brodie AM Discovery and development of Galeterone (TOK-001 or VN/124–1) for the treatment of all stages of prostate cancer. *J Med Chem* 2015; 58:2077–2087. DOI: 10.1021/jm501239f. [PubMed: 25591066]
17. Attard G, Reid AH, Auchus RJ, Hughes BA, Cassidy AM, Thompson E, Oommen NB, Folkard E, Dowsett M, Arlt W, and de Bono JS Clinical and biochemical consequences of CYP17A1 inhibition with abiraterone given with and without exogenous glucocorticoids in castrate men with advanced prostate cancer. *J Clin Endocrinol Metab* 2012 97:507–516. DOI: 10.1210/jc.2011-2189. [PubMed: 22170708]
18. Fankhauser M, Tan Y, Macintyre G, Haviv I, Hong MK, Nguyen A, Pedersen JS, Costello AJ, Hovens CM, Corcoran NM. Canonical androstenedione reduction is the predominant source of signaling androgens in hormone-refractory prostate cancer. *Clin Cancer Res* 2014; 20:5547–5557. DOI: 10.1158/1078-0432.CCR-13-3483. [PubMed: 24771644]
19. Chang KH, Li R, Papari-Zareei M, Watumull L, Zhao YD, Auchus RJ, Sharifi N. Dihydrotestosterone synthesis bypasses testosterone to drive castration-resistant prostate cancer. *Proc Natl Acad Sci U S A* 2011; 108:13728–13733. DOI: 10.1073/pnas.1107898108. [PubMed: 21795608]
20. Auchus RJ. The backdoor pathway to dihydrotestosterone. *Trends Endocrinol Metab* 2004; 15:432–438. DOI: 10.1016/j.tem.2004.09.004. [PubMed: 15519890]
21. Turcu AF, Nanba AT, Chomic R, Upadhyay SK, Giordano TJ, Shields JJ, Merke DP, Rainey WE, and Auchus RJ Adrenal-derived 11-oxygenated 19-carbon steroids are the dominant androgens in classic 21-hydroxylase deficiency. *Eur J Endocrinol* 2016; 174:601–609. DOI: 10.1530/EJE-15-1181. [PubMed: 26865584]
22. du Toit T, Bloem LM, Quanson JL, Ehlers R, Serafin AM, and Swart AC Profiling adrenal 11 β -hydroxyandrostenedione metabolites in prostate cancer cells, tissue and plasma: UPC2-MS/MS quantification of 11 β -hydroxytestosterone, 11-keto-testosterone and 11-keto-dihydrotestosterone. *J Steroid Biochem Mol Biol* 2017; 166:54–67. DOI: 10.1016/j.jsbmb.2016.06.009. [PubMed: 27345701]
23. Schiffer L, Arlt W, Storbeck KH. Intracrine androgen biosynthesis, metabolism and action revisited. *Mol Cell Endocrinol* 2018; 465:4–26. DOI: 10.1016/j.mce.2017.08.016. [PubMed: 28865807]
24. Lachance Y, Luu-The V, Labrie C, Simard J, Dumont M, de Launoit Y, Guerin S, Leblanc G, Labrie F. Characterization of human 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase gene and its expression in mammalian cells. *J Biol Chem* 1990; 265:20469–20475. [PubMed: 2243100]
25. Lachance Y, Luu-The V, Labrie C, Simard J, Dumont M, de Launoit Y, Guerin S, Leblanc G, Labrie F. Characterization of human 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4-isomerase gene and its expression in mammalian cells. *J Biol Chem* 1992; 267:3551 [PubMed: 1737804]
26. Lachance Y, Luu-The V, Verreault H, Dumont M, Rheume E, Leblanc G, Labrie F. Structure of the human type II 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase (3 beta-HSD)

- gene: adrenal and gonadal specificity. *DNA Cell Biol* 1991; 10:701–711. DOI: 10.1089/dna.1991.10.701. [PubMed: 1741954]
27. Labrie F, Simard J, Luu-The V, Belanger A, Pelletier G. Structure, function and tissue-specific gene expression of 3 β -hydroxysteroid dehydrogenase/5-ene-4-ene isomerase enzymes in classical and peripheral intracrine steroidogenic tissues. *J Steroid Biochem Mol Biol* 1992; 43:805–826. DOI: 10.1016/0960-0760(92)90308-6. [PubMed: 22217825]
 28. Labrie F, Simard J, Luu-The V, Pelletier G, Belanger A, Lachance Y, Zhao HF, Labrie C, Breton N, de Launoit Y, et al. Structure and tissue-specific expression of 3 β -hydroxysteroid dehydrogenase/5-ene-4-ene isomerase genes in human and rat classical and peripheral steroidogenic tissues. *J Steroid Biochem Mol Biol* 1992; 41:421–435. DOI: 10.1016/0960-0760(92)90368-s. [PubMed: 1562516]
 29. Labrie F, Luu-The V, Lin SX, Labrie C, Simard J, Breton R, Belanger A. The key role of 17 β -hydroxysteroid dehydrogenases in sex steroid biology. *Steroids* 1997; 62:148–158. DOI: 10.1016/S0039-128X(96)00174-2. [PubMed: 9029730]
 30. Labrie F, Luu-The V, Lin SX, Simard J, Labrie C, El-Alfy M, Pelletier G, Belanger A. Intracrinology: role of the family of 17 β -hydroxysteroid dehydrogenases in human physiology and disease. *J Mol Endocrinol* 2000; 25:1–16. DOI: 10.1677/jme.0.0250001. [PubMed: 10915214]
 31. Marchais-Oberwinkler S, Henn C, Möller G, Klein T, Negri M, Oster A, Spadaro A, Werth R, Wetzel M, Xu K, Frotscher M, Hartmann RW, Adamski J 17 β -Hydroxysteroid dehydrogenases (17 β -HSDs) as therapeutic targets: protein structures, functions, and recent progress in inhibitor development. *J Steroid Biochem* 2011; 125:66–82. DOI: 10.1016/j.jsmb.2010.12.013.
 32. Zang T, Taplin M-E, Tamae D, Xie W, Mesaris C, Zhang Z, Bubley, Montgomery B, Balk SP, Mostaghel EA, Balr IA, Penning TM Testicular vs adrenal sources of hydroxy androgens in prostate cancer. *Endocrine-Related Cancer* 2017; 24:393–404. DOI: 10.1530/ERC-17-0107. [PubMed: 28663228]
 33. Taplin ME, Montgomery B, Logothetis CJ, Bubley GJ, Richie JP, Dalkin BL, Sanda MG, Davis JW, Loda M, True LD, Troncoso P, Ye H, Lis RT, Marck BT, Matsumoto AM, Balk SP, Mostaghel EA, Penning TM, Nelson PS, Xie W, Jiang Z, Haqq CM, Tamae D, Tran N, Peng W, Kheoh T, Molina A, Kantoff PW 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; 32:3705–3715. DOI: 10.1200/JCO.2013.53.4578. [PubMed: 25311217]
 34. Locke JA, Guns ES, Lubik AA, Adomat HH, Hendy SC, Wood CA, Ettinger SL, Gleave ME, Nelson CC Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. *Cancer Res* 2008; 68:6407–6415. DOI: 10.1158/0008-5472.CAN-07-5997. [PubMed: 18676866]
 35. Locke JA, Nelson CC, Adomat HH, Hendy SC, Gleave ME, Guns ES. Steroidogenesis inhibitors alter but do not eliminate androgen synthesis mechanisms during progression to castration-resistance in LNCaP prostate xenografts. *J Steroid Biochem Mol Biol* 2009; 115:126–136. DOI: 10.1016/j.jsmb.2009.03.011. [PubMed: 19442514]
 36. Tamae DM E, Montgomery B, Nelson PS, Balk SP, Kantoff PW, Taplin ME, Penning TM The DHEA-sulfate depot following P450c17 inhibition supports the case for AKR1C3 inhibition in high risk localized and advanced castration resistant prostate cancer. *Chem Biol Interact* 2015; 234:332–338. DOI: 10.1016/j.cbi.2014.12.012. [PubMed: 25514466]
 37. Sharifi N, Auchus RJ. Androstenedione is the preferred androgen source in hormone refractory prostate cancer-Letter. *Clin Cancer Res* 2014; 20:4971 DOI: 10.1158/1078-0432.CCR-14-1123. [PubMed: 25225065]
 38. Dai C, Chung YM, Kovac E, Zhu Z, Li J, Magi-Galluzzi C, Stephenson AJ, Klein EA, Sharifi N. Direct Metabolic Interrogation of Dihydrotestosterone Biosynthesis from Adrenal Precursors in Primary Prostatectomy Tissues. *Clin Cancer Res* 2017; 23:6351–6362. DOI: 10.1158/1078-0432.CCR-17-1313. [PubMed: 28733443]
 39. Stuchbery R, McCoy PJ, Hovens CM, Corcoran NM Androgen synthesis in prostate cancer: do all roads lead to Rome? *Nat Rev Urol* 2017; 14:49–58. DOI: 10.1038/nrurol.2016.221. [PubMed: 27824348]

40. Deb S, Pham S, Ming DS, Chin MY, Adomat H, Hurtado-Coll A, Gleave ME, Guns EST Characterization of Precursor-Dependent Steroidogenesis in Human Prostate Cancer Models. *Cancers (Basel)* 2018; 10:pii: E343 DOI: 10.3390/cancers10100343. [PubMed: 30241348]
41. Zhu Z, Chung YM, Sergeeva O, Kepe V, Berk M, Li J, Ko HK, Li Z, Petro M, DiFilippo FP, Lee Z, Sharifi N. Loss of dihydrotestosterone-inactivation activity promotes prostate cancer castration resistance detectable by functional imaging. *J Biol Chem* 2018; 293:17829–17837. DOI: 10.1074/jbc.RA118.004846. [PubMed: 30262668]
42. Pfeiffer MJ, Smit FP, Sedelaar JP, and Schalken JA Steroidogenic enzymes and stem cell markers are upregulated during androgen deprivation in prostate cancer. *Mol Med* 2011; 17:657–664. DOI: 10.2119/molmed.2010.00143. [PubMed: 21365123]
43. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, Febbo PG, and Balk SP Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Res* 2006; 66:2815–2825. DOI: 10.1158/0008-5472.CAN-05-4000. [PubMed: 16510604]
44. Gauthier-Landry L, Bélanger A, Barbier O Multiple roles for UDP-glucuronosyltransferase (UGT)2B15 α and UGT2B17 enzymes in androgen metabolism and prostate cancer evolution. *J Steroid Biochem Mol Biol* 2015; 145:187–192. DOI: 10.1016/j.jsbmb.2014.05.009. [PubMed: 24861263]
45. Arakawa H., Nakanishi T, Yanagihara C, Nishimoto T., Wakayama T, Mizokami A, Namiki M, Kawa K, Tamai I Enhanced expression of organic anion transporting polypeptides (OATPs) in androgen receptor-positive prostate cancer cells: possible role of OATP1A2 in adaptive cell growth under androgen-depleted conditions. *Biochem Pharmacol* 2012; 84:1070–1077. DOI: 10.1016/j.bcp.2012.07.026. [PubMed: 22864060]
46. Cho E, Montgomery RB, and Mostaghel EA., Minireview: SLCO and ABC transporters: a role for steroid transport in prostate cancer progression. *Endocrinology* 2014; 155:4124–4132. DOI: 10.1210/en.2014-1337. [PubMed: 25147980]
47. Green SM, Kaipainen A, Bullock K, Zhang A, Lucas JM, Matson C, Banks WA, Mostaghel EA Role of OATP transporters in steroid uptake by prostate cancer cells in vivo. *Prostate Cancer Prostatic Dis* 2017; 20:20–27. DOI: 10.1038/pcan.2016.42. [PubMed: 27645128]
48. Wright JL, Kwon EM, Ostrander EA, Montgomery RB, Lin DW, Vessella R, Stanford JL, EA M Expression of SLCO transport genes in castration-resistant prostate cancer and impact of genetic variation in SLCO1B3 and SLCO2B1 on prostate cancer outcomes. *Cancer Epidemiol Biomarkers Prev* 2011; 20:619–627. DOI: 10.1158/1055-9965. [PubMed: 21266523]
49. Farnsworth WE. Human prostatic dehydroepiandrosterone sulfate sulfatase. *Steroids* 1973; 21:647–664. DOI: 10.1016/0039-128x(73)90134-7. [PubMed: 4267483]
50. Klein H, Molwitz T, W. B Steroid sulfate sulfatase in human benign prostatic hyperplasia: characterization and quantification of the enzyme in epithelium and stroma. *J Steroid Biochem* 1989; 33:195–200. DOI: 10.1016/0022-4731(89)90294-x. [PubMed: 2475673]
51. Selcer KW, Kabler H, Sarap J, Xiao Z, Li P-K. Inhibition of steryl sulfatase activity in LNCaP human prostate cancer cells. *Steroids* 2002; 67:821–826. DOI: 10.1016/s0039-128x(02)00030-2. [PubMed: 12231117]
52. Dalla Valle L, Toffolo V, Nardi A, Fiore C, Armanini D, Belvedere P, Colombo L. The expression of the human steroid sulfatase-encoding gene is driven by alternative first exons. *J Steroid Biochem Mol Biol* 2007; 107:22–29. DOI: 10.1016/j.jsbmb.2007.05.004. [PubMed: 17601726]
53. Chang KH, Li R, Kuri B, Lotan Y, Roehrborn CG, Liu J, Vessella R, Nelson PS, Kapur P, Guo X, Mirzaei H, Auchus RJ, Sharifi N. A gain-of-function mutation in DHT synthesis in castration-resistant prostate cancer. *Cell* 2013; 154:1074–1084. DOI: 10.1016/j.cell.2013.07.029. [PubMed: 23993097]
54. Sabharwal N, Sharifi N HSD3B1 Genotypes conferring adrenal restrictive and adrenal permissive phenotypes on prostate cancer and beyond. *Endocrinology* 2019; 10.1210/en.2019-00366.
55. Li Z, Alyamani M, Li J, Rogacki K, Abazeed M, Upadhyay SK, Balk SP, Taplin ME, Auchus RJ, Sharifi N. Redirecting abiraterone metabolism to fine-tune prostate cancer anti-androgen therapy. *Nature* 2016; 533:547–551. DOI: 10.1038/nature17954. [PubMed: 27225130]

56. Li Z, Bishop AC, Alyamani M, Garcia JA, Dreicer R, Bunch D, Liu J, Upadhyay SK, Auchus RJ, Sharifi N. Conversion of abiraterone to D4A drives anti-tumour activity in prostate cancer. *Nature* 2015; 523:347–351. DOI: 10.1038/nature14406. [PubMed: 26030522]
57. Li R, Evaul K, Sharma KK, Chang KH, Yoshimoto J, Liu J, Auchus RJ, Sharifi N. Abiraterone inhibits 3beta-hydroxysteroid dehydrogenase: a rationale for increasing drug exposure in castration-resistant prostate cancer. *Clin Cancer Res* 2012; 18:3571–3579. DOI: 10.1158/1078-0432.CCR-12-0908. [PubMed: 22753664]
58. McKay RR, Werner L, Mostaghel EA, Lis R, Voznesensky O, Zhang Z, Marck BT, Matsumoto AM, Domachevsky L, Zukotynski KA, Bhasin M, Bubley, Montgomery B, Kantoff PW, Balk SP, Taplin ME A Phase II Trial of Abiraterone Combined with Dutasteride for Men with Metastatic Castration-Resistant Prostate Cancer. *Clin Cancer Res* 2017; 23:935–945. DOI: 10.1158/1078-0432.CCR-16-0987. [PubMed: 27683182]
59. Luu-The V, Zhang Y, Poirier D, Labrie F. Characteristics of human types 1, 2 and 3, 17 beta-hydroxysteroid dehydrogenase activities: oxidation/reduction and inhibition. *J Steroid Biochem Mol Biol* 1995; 55:581–587. DOI: 10.1016/0960-0760(95)00209-x. [PubMed: 8547185]
60. Jin JZ, Lin SX Human estrogenic 17beta-hydroxysteroid dehydrogenase: predominance of estrone reduction and its induction by NADPH. *Biochem Biophys Res Commun* 1999; 259:489–493. DOI: 10.1006/bbrc.1999.0704. [PubMed: 10362535]
61. Miettinen MM, Poutanen MH, Vihko RK Characterization of estrogen-dependent growth of cultured MCF-7 human breast-cancer cells expressing 17beta-hydroxysteroid dehydrogenase type 1. *Int J Cancer* 1996; 68:600–604. DOI: 10.1002/(SICI)1097-0215(19961127)68:5<600::AID-IJC8>3.0.CO;2-2. [PubMed: 8938141]
62. Andersson S, Geissler WM, Patel S, and Wu L The molecular biology of androgenic 17b-hydroxysteroid dehydrogenases. *J Steroid Biochem Mol Biol* 1995; 53:37–39. DOI: 10.1016/0960-0760(95)00039-3. [PubMed: 7626483]
63. Lin H-K, Jez JM, Schlegel BP, Peehl DM, Pachter JA and Penning TM Expression and characterization of recombinant type 2 3a-hydroxysteroid dehydrogenase (HSD) from human prostate: demonstration of bifunctional 3a/17b-HSD activity and cellular distribution. *Mol Endocrinol* 1997; 11:1971–1984. DOI: 10.1210/mend.11.13.0026. [PubMed: 9415401]
64. Hofland J, van Weerden WM, Dits NFJ, Steenbergen J, van Leenders GH, Jenster G, Schroder FH, and de Jong FH Evidence of limited contributions for intratumoral steroidogenesis in prostate cancer. *Cancer Res* 2010; 70:1256–1264. DOI: 10.1158/0008-5472.CAN-09-2092. [PubMed: 20086173]
65. Hamid AR, Pfeiffer MJ, Verhaegh GW, Schaafsma E, Brandt A, Sweep FC, Sedelaar JP, and Schalken JA Aldo-keto reductase family 1 member C3 (AKR1C3) is a biomarker and therapeutic target for castration-resistant prostate cancer. *Mol Med* 2012; 18:1449–1455. DOI: 10.2119/molmed.2012.00296.
66. Mitsiades N, Sung CC, Schultz N, Danila DC, He B, Eedunuri VK, Fleisher M, Sander C, Sawyers CL and Scher HI Distinct patterns of dysregulated expression of enzymes involved in androgen synthesis and metabolism in metastatic prostate cancer tumors. *Cancer Res* 2012; 72:6142–6152. DOI: 10.1158/0008-5472.CAN-12-1335. [PubMed: 22971343]
67. Lin HK, Steckelbroeck S, Fung KM, Jones AN, and Penning TM Characterization of a monoclonal antibody for human aldo-keto reductase AKR1C3 (type 2 3a-hydroxysteroid dehydrogenase/type 5 17b-hydroxysteroid dehydrogenase); immunohistochemical detection in breast and prostate. *Steroids* 2004; 69:795–801. DOI: 10.1016/j.steroids.2004.09.014. [PubMed: 15582534]
68. Jernberg E, Thysell E, Ylitalo EB, Rudolfsson S, Crnalic S, Widmark A, Bergh A, Wikstrom P Characterization of the prostate cancer bone metastases according to expression levels of steroidogenic enzymes and androgen receptor splice variants. *PLOS One* 2013; 8:e77407 DOI: 10.1371/journal.pone.0077407. [PubMed: 24244276]
69. Powell KSL, Conley-LaComb MK, Asangani I, Wu YM, Ginsburg KB, Williams J, Squire JA, Maddipati KR, Cher ML, Chinni SR. ERG/AKR1C3/AR constitutes a feed-forward loop for AR signaling in prostate Cancer Cells. *Clin Cancer Res* 2015; 21:2569–2579. DOI: 10.1158/1078-0432.CCR-14-2352. [PubMed: 25754347]

70. Tian Y, Zhao L, Zhang H, Liu X, Zhao L, Zhao X and Li J. AKR1C3 overexpression may serve as a promising biomarker for prostate cancer progression. *Diagnostic Pathol* 2014; 9:42–48. DOI: 10.1186/1746-1596-9-42.
71. Liu C, Armstrong CM, Lou W, Lombard A, Evans CP, Gao AC. Inhibition of AKR1C3 Activation Overcomes Resistance to Abiraterone in Advanced Prostate Cancer. *Mol Cancer Ther* 2017; 16:35–44. DOI: 10.1158/1535-7163.MCT-16-0186. [PubMed: 27794047]
72. Liu CLW, Zhu Y, Yang JC, Nadiminty N, Gaikwad NW, Evans CP, Gao AC. Intracrine androgens and AKR1C3 activation confer resistance to Enzalutamide in prostate cancer. *Cancer Res* 2015; 75:1413–1422. DOI: 10.1158/0008-5472.CAN-14-3080. [PubMed: 25649766]
73. Platt A, Xia Z, Liu Y, Chen G, Lazarus P. Impact of nonsynonymous single nucleotide polymorphisms on in-vitro metabolism of exemestane by hepatic cytosolic reductases. *Pharmacogenet Genomics* 2016; 26:370–380. DOI: 10.1097/FPC.0000000000000226. [PubMed: 27111237]
74. Gao X, Dai C, Huang S, Tang J, Chen G, Li J, Zhu Z, Zhu X, Zhou S, Gao Y, Hou Z, Fang Z, Xu C, Wang J, Wu D, Sharifi N, Li Z. Functional Silencing of HSD17B2 in Prostate Cancer Promotes Disease Progression. *Clin Cancer Res* 2019; 25:1291–1301. DOI: 10.1158/1078-0432.CCR-18-2392. [PubMed: 30228209]
75. Ko HK, Berk M, Chung YM, Willard B, Bareja R, Rubin M, Sboner A, Sharifi N. Loss of an Androgen-Inactivating and Isoform-Specific HSD17B4 Splice Form Enables Emergence of Castration-Resistant Prostate Cancer. *Cell Rep* 2018; 22:809–819. DOI: 10.1016/j.celrep.2017.12.081. [PubMed: 29346776]
76. Bauman DR, Steckelbroeck S, Peehl DM and Penning TM Transcript profiling of the androgen signal in normal prostate, benign prostatic hyperplasia, and prostate cancer. *Endocrinology* 2006; 147:5806–5816. DOI: 10.1210/en.2006-0627. [PubMed: 16959841]
77. Rizner T, Lin H-K, Peehl DM, Steckelbroeck S, Bauman DR and Penning TM Human type 3 3 α -hydroxysteroid dehydrogenase (AKR1C2) and androgen metabolism in prostate cells. *Endocrinology* 2003; 144:2922–2932. DOI: 10.1210/en.2002-0032. [PubMed: 12810547]
78. Takahashi R, Grigliatti TA, Reid RE, Riggs KW. The effect of allelic variation in aldo-keto reductase 1C2 on the in vitro metabolism of dihydrotestosterone. *J Pharmacol Exp Therapeutics* 2009; 329:1032–1039. DOI: 10.1124/jpet.109.150995.
79. Steckelbroeck S, Jin Y, Gopishetty S, Oyesanmi B, and Penning TM Human cytosolic 3 α -hydroxysteroid dehydrogenases of the aldo-keto reductase superfamily display significant 3 β -hydroxysteroid dehydrogenase activity: Implications for steroid hormone metabolism and action. *J Biol Chem* 2003; 279:10784–10795. DOI: 10.1074/jbc.M313308200. [PubMed: 14672942]
80. Bauman DR, Steckelbroeck S, Williams MV, Peehl DM, Penning TM. 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; 20:444–458. DOI: 10.1210/me.2005-0287. [PubMed: 16179381]
81. Mohler JL, Titus MA, Bai S, Kennerley BJ, Lih FB, Tomer KB, Wilson EM. Activation of the androgen receptor by intratumoral bioconversion of androstenediol to dihydrotestosterone in prostate cancer. *Cancer Res* 2011; 71:1486–1496. DOI: 10.1158/0008-5472.CAN-10-1343. [PubMed: 21303972]
82. Mohler JL, Titus MA, Wilson EM. Potential prostate cancer drug target: bioactivation of androstenediol by conversion to dihydrotestosterone. *Clin Cancer Res* 2011; 17:5844–5849. DOI: 10.1158/1078-0432.CCR-11-0644. [PubMed: 21705451]
83. Thomas MP, Potter BV Estrogen O-sulfamates and their analogues: Clinical steroid sulfatase inhibitors with broad potential. *J Steroid Biochem Mol Biol* 2015; 153:160–169. DOI: 10.1016/j.jsbmb.2015.03.012.
84. Takahashi M, Luu-The V, Labrie F. Inhibitory effect of synthetic progestins, 4-MA and cyanoketone on human placental 3 β -hydroxysteroid dehydrogenase/5 α →4-ene-isomerase activity. *J Steroid Biochem Mol Biol* 1990; 37:231–236. DOI: 10.1016/0960-0760(90)90331-e. [PubMed: 2268554]

85. Thomas JL, Bucholtz KM, Sun J, Mack VL, Kacsoh B. Structural basis for the selective inhibition of human 3 β -hydroxysteroid dehydrogenase 1 in human breast tumor MCF-7 cells. *Mol Cell Endocrinol* 2009; 301:174–182. DOI: 10.1016/j.mce.2008.09.029. [PubMed: 18955108]
86. Thomas JL, Mack VL, Glow JA, Moshkelani D, Terrell JR, Bucholtz KM. Structure/function of the inhibition of human 3 β -hydroxysteroid dehydrogenase type 1 and type 2 by trilostane. *J Steroid Biochem Mol Biol* 2008; 111:66–73. DOI: 10.1016/j.jsbmb.2008.04.007. [PubMed: 18524572]
87. Fiandalo MV, Gewirth DT, Mohler JL. Potential impact of combined inhibition of 3 α -oxidoreductases and 5 α -reductases on prostate cancer. *Asian J Urol* 2019; 6:50–56. DOI: 10.1016/j.ajur.2018.09.002. [PubMed: 30775248]
88. Barnard M, Quanson JL, Mostaghel E, Pretorius E, Snoep JL, Storbeck KH. 11-Oxygenated androgen precursors are the preferred substrates for aldo-keto reductase 1C3 (AKR1C3): Implications for castration resistant prostate cancer. *J Steroid Biochem Mol Biol* 2018; 183:192–201. DOI: 10.1016/j.jsbmb.2018.06.013. [PubMed: 29936123]
89. Bauman DR, Rudnick S, Szewczuk L, Sridhar G and Penning TM Development of nonsteroidal anti-inflammatory drug analogs and steroid carboxylates selective for human aldo-keto reductase isoforms: potential antineoplastic agents that work independently of cyclooxygenase isozymes. *Mol Pharmacol* 2005; 67:60–68. DOI: 10.1124/mol.104.006569. [PubMed: 15475569]
90. Byrns MC, Steckelbroeck S and 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; 75:484–493. DOI: 10.1016/j.bcp.2007.09.008. [PubMed: 17950253]
91. Liedtke AJ, Adeniji AO, Chen M, Byrns MC, Jin Y, Christianson DW, Marnett LJ and Penning TM Development of potent and selective indomethacin analogues for the inhibition of AKR1C3 (type 5 17 β -hydroxysteroid dehydrogenase/prostaglandin F synthase) in castrate-resistant prostate cancer. *J Med Chem* 2013; 56:2429–2446. DOI: 10.1021/jm3017656. [PubMed: 23432095]
92. Adeniji AO, Twenter BM, Byrns MC, Jin Y, Winkler JD, and Penning TM Discovery of substituted 3-(phenylamino)benzoic acids as potent and selective inhibitors of type 5 17 β -hydroxysteroid dehydrogenase (AKR1C3). *Bioorg Med Chem Lett* 2011; 21:1464–1468. DOI: 10.1016/j.bmcl.2011.01.010. [PubMed: 21277203]
93. Adeniji AO, Twenter BM, Byrns MC, Jin Y, Chen M, Winkler JD, and Penning TM 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; 55:2311–2323. DOI: 10.1021/jm201547v. [PubMed: 22263837]
94. Adeniji A, Uddin MJ, Zang T, Tamae D, Wangtrakuldee P, Marnett LJ, and Penning TM Discovery of (R)-2-(6-Methoxynaphthalen-2-yl)butanoic Acid as a Potent and Selective Aldo-keto Reductase 1C3 Inhibitor. *J Med Chem* 2016; 59:7431–7444. DOI: 10.1021/acs.jmedchem.6b00160. [PubMed: 27486833]
95. Kikuchi A, Furutani T, Azami H, Watanbe K, Nimi T, Kamiyama Y, Kuromitsu S, Bakin-Bey E, Heeringa M, Uoatas T, and Enjo K In vitro and in vivo characterization of ASP9521: a novel selective, orally bioavailable inhibitor of 17 β -hydroxysteroid dehydrogenase type 5 (17 β -HSD5; AKR1C3). *Invest New Drugs* 2014; 32:860–870. DOI: 10.1007/s10637-014-0130-5. [PubMed: 24981575]
96. Lorient Y, Fizazi K, Jones RJ, Brand Van den J, Molife RL, Omlin A, James ND, Baskin-Bey E, Heeringa M, Baron B, Holtkamp GM, Ouatas T, and de Bono JS Safety, tolerability and anti-tumor activity of the androgen biosynthesis inhibitor ASP9521 in patients with metastatic castration-resistant prostate cancer: multi-centre phase I/II study. *Invest New Drugs* 2014; 32:995–1004. DOI: 10.1007/s10637-014-0101-x. [PubMed: 24771350]
97. Yepuru M, Wu Z, Kyulkarni A, Yin F, Barrett CM, Kim J, Steiner MS, Miller DD, Dalton JT and Narayanan R Steroidogenic enzyme AKR1C3 is a novel androgen receptor-selective coactivator that promotes prostate cancer growth. *Clin Cancer Res* 2013; 19:5613–5625. DOI: 10.1158/1078-0432.CCR-13-1151. [PubMed: 23995860]
98. Wangtrakuldee P, Adeniji AO, Zang T, Duan L, Khatri B, Twenter BM, Estrada MA, Higgins TF, Winkler JD, Penning TM A 3-(4-nitronaphthen-1-yl) amino-benzoate analog as a bifunctional

- AKR1C3 inhibitor and AR antagonist: Head to head comparison with other advanced AKR1C3 targeted therapeutics. *J Steroid Biochem & Mol Biol* 2019; 10.1016/j.jsbmb.2019.01.001.
99. Byrns MC, Mindnich R, Duan L, and Penning TM Overexpression of aldo-keto reductase 1C3 (AKR1C3) in LNCaP cells diverts androgen metabolism towards testosterone resulting in resistance to the 5 α -reductase inhibitor finasteride. *J Steroid Biochem Mol Biol* 2012; 130:7–15. DOI: 10.1016/j.jsbmb.2011.12.012. [PubMed: 22265960]
100. Montgomery RB, Mostaghel EA, Vessella R, Hess DL, Kalhorn TF, Higano CS, True LD Nelson PS Maintenance of Intratumoral Androgens in Metastatic Prostate Cancer: A Mechanism for Castration-Resistant Tumor Growth. *Cancer Res* 2008; 68:4447–4454. DOI: 10.1158/0008-5472.CAN-08-0249. [PubMed: 18519708]
101. Lacy JM, Kyprianou N. A tale of two trials: The impact of 5 α -reductase inhibition on prostate cancer (Review. *Oncol Lett* 2014; 8:1391–1396. DOI: 10.3892/ol.2014.2388. [PubMed: 25202340]
102. Puhr M, Hoefer J, Eigentler A, Ploner C, Handle F, Schaefer G, Kroon J, Leo A, Heidegger I, Eder I, Culig Z, Van der Pluijm G, Klocker H The Glucocorticoid Receptor Is a Key Player for Prostate Cancer Cell Survival and a Target for Improved Antiandrogen Therapy. *Clin Cancer Res* 2018; 24:927–938. DOI: 10.1158/1078-0432.CCR-17-0989. [PubMed: 29158269]

Highlights

- Target tissues synthesize steroid hormones that is independent of the gonadal-hypothalamic pituitary axis
- The intracrine formation of steroid hormones in prostate cancer occurs by several pathways
- Interrogation of these pathways should be performed using mass spectrometry
- Relevant human serum concentrations of precursors should be used to trace metabolic pathways
- Pathways should be interrogated under conditions that mimic androgen deprivation therapy

Figure 1.

Androgen metabolism in human prostate. The canonical pathway is shown in red; the alternative pathway is shown in blue; the backdoor pathway is shown in pink; and the pathway from 5-androstenediol is shown in black. Enzymes are identified by their gene names which are italicized.

Figure 2. 11-Oxo-Androgens. Formation of 11-oxo-T and 11-oxo-DHT from their 11β-hydroxysteroid precursors. Enzymes are identified by their gene names which are italicized.

Fig 3.

Overexpression of AKR1C3 in CRPC. RT-PCR, and Affymetrix microarray detection of AKR1C3 RNA expression in normal prostate (Pr) and androgen-independent prostate cancer (AIPC-also known as castration resistant prostate cancer, CRPC), Panel A; specificity of murine anti-human AKR1C3 antibody for recombinant AKR1C3 versus its highly related isoforms AKR1C1, AKR1C2 and AKR1C4, Panel B; immunohistochemical detection of AKR1C3 in CRPC (top left) and staining with pre-immune serum (top-right); immunohistochemical detection of AKR1C3 in soft tissue metastatic tissue, (bottom left) and staining with pre-immune serum (bottom right); Taken from references (43,67).

Fig. 4.
Position of nsSNPs in the AKR1C3 crystal structure. NADP+ (green); indomethacin, a competitive inhibitor (magenta), nsSNPs (in red), Calpha-chain in blue. Created in PyMol. Taken from PDB 1S2A

Table 1.**Human 17 β -Hydroxysteroid Dehydrogenase Isoforms**

Type #	Gene	SDR/AKR Nomenclature	Other Aliases/Descriptors
1	HSD17B1	SDR28C1	Estrogenic 17 β -hydroxysteroid dehydrogenase Reduces E1 to E2, 4-AD to T
2	HSD17B2	SDR9C2	Oxidizes E2 to E1, T to 4-AD and 20 α -hydroxyprogesterone to progesterone
3	HSD17B3	SDR12C2	Reduces 4-AD to T and is Leydig cell specific
4	HSD17B4	SDR8C1	Bifunctional enzyme involved in peroxisomal β -oxidation of fatty acids
5	AKR1C3	AKR1C3	Prostaglandin F synthase and dihydrodiol dehydrogenase X. Reduces 4-AD to T; 5 α -Adione to 5 α -DHT; E1 to E2; and Progesterone to 20 α -hydroxyprogesterone
6	HSD17B6	SDR9C6	RL-HSD, RODH like 3 α -HSD Oxidizes 3 α -Adiol to 5 α -DHT; also acts as a 3 α -HSD epimerase
7	HSD17B7	SDR37C1	Reduces E1 to E2 in breast cancer cells and acts as a 3-ketosteroid reductase in cholesterol biosynthesis
8	HSD17B8	SDR30C1	Subunit of ketoacyl reductase 1 of mitochondrial fatty acid synthase. Oxidizes E2, T and 5 α -DHT
9	HSD17B9	SDR9C5-2	Retinol dehydrogenase 5 (RDH5, RODH5) Biosynthesis of 11- <i>cis</i> -retinaldehyde
10	HSD17B10	SDR5C1	ERAB, short chain L-3-hydroxyacyl-CoA dehydrogenase dehydrogenase. oxidation of fatty acids and steroids
11	HSD17B11	SDR16C2	Short chain alcohol dehydrogenase, metabolize ketones and alcohols
12	HSD17B12	SDR12C1	Reduces E1 to E2 in ovarian tissues; very long chain fatty acid elongation
13	HSD17B13	SDR16C3	Human liver lipid droplet associated protein involved in pathogenesis of NAFLD
14	HSD17B14	SDR47C1	Oxidizes E2 to E1, and 5 α -Adiol to DHEA Expression in kidney, ovaries, and other tissues

E1 = estrone; E2 = 17 β -estradiol; 4-AD, = 4-androstene-3,17-dione; T= testosterone; 5 α -Adione= 5 α -androstane-3,17-dione; 5 α -DHT= 5 α -dihydrotestosterone; DHEA = dehydroepiandrosterone; 5-Adiol = 5-androstene-3 β , 17 β -diol.

Table 2.

Prevailing Concentrations of Serum Androgens in Castrate Males

Steroid	Intact (ng/dL)	Castrate (ng/dL)
Testosterone *	336 [214–691]	11.2 [4.7–18.5]
Epitestosterone *	< 1.0	<1.0
Dihydrotestosterone *	33.2 [18.7–71.1]	3.3 [<1.0– 10.4]
Dehydroepiandrosterone *	206 [126 – 392]	227 [177–330]
Androsterone *	17.6 [4.9–35.6]	8.6 [2.7–22.0]
Epiandrosterone *	6.0 [2.5–16]	5.3 [2.5–17.0]
4-Androstene-3,17-dione **	76	52
Testosterone-G *	99.8 [42.1– 71.3]	19.7 [7.3–69.7]
Epitestosterone-G *	<2.6	<2.6
Dihydrotestosterone-G *	34.4 [15.3–68.8]	10.9 [<2.6 – 27.8]
Dehydroepiandrosterone-G *	108.5 [45.0– 188.1]	82.2 [31.9–224.5]
Androsterone-G *	2500 [1666–3156]	1500 [1043 –2934]
Testosterone-SO ₄ *	<204 (LLOD)	<204 (LLOD)
Epitestosterone-SO ₄ *	<204 (LLOD)	<204 (LLOD)
Dihydrotestosterone-SO ₄ *	<204 (LLOD)	<204 (LLOD)
Dehydroepiandrosterone-SO ₄ *	166,000 [121,000 – 259,000]	210,000 [115,000 – 375,000]
Androsterone-SO ₄ *	<204 (LLOD)	<204 (LLOD)
11β-Hydroxy- 4-Androstene-3,17-dione ***	ND	12,000
11-Oxo- 4-Androstene-3,17-dione ***	ND	0.75 < LLQ
11β-Hydroxytestosterone ***	ND	0.30<LLQ
11-Oxo-testosterone ***	ND	12,000
11β-Hydroxy-5α-dihydrotestosterone	ND	ND
11-Oxo-5α-dihydrotestosterone ***	ND	6.0<LLQ

Rows in yellow identify a significant decrease due to castration.

* Taken from (32)

** Taken from (33)

*** Taken from (22); values from a single patient

ND = not determined

Table 3.Levels of DHEA-SO₄ following inhibition of *CYP17A1* by Abiraterone in CRPC Patients

	12 weeks Leuprolide followed by 12 weeks Leuprolide + Abiraterone Acetate + Prednisone. Median Serum androgen level (ng/dL)			
Steroid	Base Line (n =28)	Week 12 (n= 28)	Week 24 (n =28)	P value
Testosterone	429	17	5	0.003
DHT	29	13	17	0.0176
DHEA	242	201	19	<0.0001
DHEA-Glucuronide	1901	1508	515	0.0003
DHEA-SO₄	231,000	200,000	22,000	<0.0001
Androsterone	11	5	0.7	0.0003
4 Androstene-3,17-dione	76	52	7	<0.0001

Taken from ref (33,36).