Published in final edited form as:

Vitam Horm. 2018 ; 108: 309–331. doi:10.1016/bs.vh.2018.01.007.

Dehydroepiandrosterone (DHEA)-SO₄ Depot and Castration-Resistant Prostate Cancer

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Abstract

Dehydroepiandrosterone (DHEA)-SO₄ of adrenal origin is the major C19 steroid in the serum. It is a precursor of intratumoral androgen biosynthesis in patients with advanced prostate cancer following chemical or surgical castration. DHEA is a product of the P450c17 (17 α -hydroxylase-17,20-lyase) enzyme. Despite inhibition of P450c17 with new agents, e.g., Abiraterone acetate, Orterenol, and Galeterone, the level of enzyme inhibition rarely exceeds 90% leaving behind a significant depot for androgen biosynthesis within the tumor. For DHEA-SO₄ to be utilized there is uptake by organic anion transporter polypeptides, deconjugation catalyzed by steroid sulfatase, and adaptive upregulation of prostate steroidogenic enzymes that will convert DHEA into either testosterone or dihydrotestosterone. The depot of DHEA-SO₄ that remains after P450c17 inhibition and the adaptive responses that occur within the tumor to promote DHEA utilization contribute to mechanisms of drug resistance observed with P450c17 inhibitors. Knowledge of these mechanisms identify new targets for therapeutics that could be used to surmount drug resistance in prostate cancer.

1. INTRODUCTION

Prostate cancer is the leading cause of cancer in males in the United States and has the second highest mortality. Approximately 161,000 new cases are diagnosed annually resulting in 27,000 deaths. It is estimated that more than 3.0 million men in the United States live with prostate cancer (SEER, 2017). Advanced localized prostate cancer is androgen dependent and Huggins and coworkers showed that surgical castration, followed by adrenal-ectomy and replacement glucocorticoids led to remission (Huggins, 1965; Huggins, Stevens, & Hodges, 1941). This surgery has been supplanted by chemical castration which consists of administration of a LH-RH agonist, e.g., Leuprolide (Sharifi et al., 1996) which is often coadministered with an androgen receptor (AR) antagonist (*R*-bicalutamide also known as Casodex) (Furr et al., 1987). This first-line androgen deprivation therapy (ADT) leads to remission of the cancer but invariably there is relapse and “castration-resistant prostate cancer (CRPC),” the lethal form of the disease emerges.

CRPC is accompanied by a rising serum prostatic-specific antigen (PSA). PSA levels increase as a result of androgen induction of the *KLK3* gene which occurs in a background

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of castrate levels of circulating testosterone (T). This phenomenon occurs since the tumor adapts to ADT to reactivate androgen signaling. Androgen signaling is reactivated by intracrine androgen biosynthesis and by changes in the AR itself (Knudsen & Penning, 2010; Knudsen & Scher, 2009). Intratumoral androgen biosynthesis can occur by either de novo steroidogenesis starting from cholesterol, or by conversion of adrenal androgens, e.g., dehydroepiandrosterone (DHEA)-SO₄ and DHEA into T and 5 α -dihydrotestosterone (DHT). This chapter will examine the role of DHEA-SO₄ and DHEA as a major source of tumor androgens where the former is the most abundant steroid in the male circulation and is present in a 400- to 1000-fold excess over DHEA (Damgaard-Olesen et al., 2016; Shackleton, Kletke, Wudy, & Pratt, 1990; Tamae et al., 2013).

2. INTRACRINE FORMATION OF ANDROGENS USING DHEA AS A PRECURSOR

The concept that DHEA-SO₄ and DHEA could be important precursors of prostatic testosterone T and DHT was originally proposed by Labrie and coworkers (Labrie et al., 1998; Labrie, Bélanger, Simard, Luu-The, & Labrie, 1995). DHEA is synthesized in the zona reticularis of the adrenal cortex from pregnenolone which is converted to 17 α -hydroxypregnenolone via the 17 α -hydroxylase activity catalyzed by P450c17. The resultant 17 α -hydroxypregnenolone is converted to DHEA via the 17,20-lyase activity of the same enzyme (Chung et al., 1987; Nakajin, Shinoda, Haniu, Shively, & Hall, 1984), Fig. 1. The electron donor for P450c17 is NADPH cytochrome P450 oxidoreductase (Estabrook, Shet, Fisher, Jenkins, & Waterman, 1996), even though the activity of the lyase is determined by the availability of cytochrome *b5* (Auchus, Lee, & Miller, 1998; Peng et al., 2016). In the absence of cytochrome *b5*, 17 α -hydroxypregnenolone is converted to glucocorticoids in the zona fasciculata. Thus, cytochrome *b5* is the molecular switch that determines whether the 17,20-lyase activity will proceed. Once formed a significant portion of DHEA is sulfonated by SULT2A1 (hydroxysteroid sulfotransferase) to form DHEA-SO₄ (Saner et al., 2005). DHEA-SO₄ is then transported into the prostate by organic anion transporter polypeptides (OATPs), e.g., encoded by *SLCO1A2*, *SLCO1B1*, *SLCO1B3*, and *SLCO2B1*, (Arakawa et al., 2012; Cho, Montgomery, & Mostaghel, 2014), deconjugated by steroid sulfatase (STS) and then converted to T and DHT.

The conversion of DHEA to T and DHT can occur by three routes. In the first route, DHEA is converted to 4-androstene-3,17-dione (4-AD) by 3 β -hydroxysteroid dehydrogenase type 1 (HSD3B1), which is reduced by 17 β -hydroxysteroid dehydrogenase type 5 (AKR1C3) to yield T, which is further reduced by steroid 5 α -reductase (SRD5A) to yield DHT (this route is known as the canonical pathway) (Fankhauser et al., 2014); in the second route, 4-AD is reduced by SRD5A to yield 5 α -androstane-3,17-dione (5-Adione) and then reduced by AKR1C3 to yield DHT (in this alternative pathway, T is by-passed altogether) (Chang et al., 2011); and in the third route, DHEA is reduced by AKR1C3 to yield 5-androstene-3 β ,17 β -diol (5-Adiol), which is then converted by HSD3B1 to yield T, which can be further reduced to DHT. Each of these steps will be discussed in greater detail in subsequent sections.

DHEA can also be by-passed in the back door pathway to prostatic androgens (Andriole et al., 2004; Auchus, 2004; Mohler, Titus, Bai, et al., 2011; Mohler, Titus, & Wilson, 2011). In this pathway, pregnenolone of adrenal origin is converted to progesterone which is reduced to 5 α -dihydroprogesterone by SRD5A, and subsequently reduced to allopregnanolone and then converted to androsterone by P450c17. Androsterone is then reduced to 5 α -androstane-3 α ,17 β -diol (3 α -diol) by AKR1C3, which is oxidized by HSD17B6 to DHT.

3. ADT FOR ADVANCED PROSTATE CANCER

Standard treatment of care for prostate cancer using first-line ADT has evolved to aggressively suppress all sources of androgen by which the tumor can synthesize its own T and DHT. Starting with Leuprolide additional agents have been added to the regimen, including *R*-bicalutamide and dutasteride (a dual inhibitor of steroid 5 α -reductase type 1 and type 2) (Andriole et al., 2004), and ketoconazole (a P450c17 inhibitor) (Kühn-Velten & Lessmann, 1992; Trachtenberg, Halpern, & Pont, 1983).

3.1 Targeted Androgen Pathway Suppression

The effect of combined first-line ADT was assessed in a targeted androgen pathway suppression (TAPS) clinical trial (Mostaghel et al., 2014). Patients with localized prostate cancer were randomized into four groups: group 1 patients received goserelin (LH-RH agonist) plus bicalutamide; group 2 patients received goserelin plus dutasteride; group 3 patients received goserelin, bicalutamide, and dutasteride; and group 4 patients received goserelin, bicalutamide, dutasteride, and ketoconazole. All treatment arms showed a statistically significant reduction in prostate tissue DHT, but the largest change was observed in group 4, which also showed the largest change in serum T, androsterone, and DHEA-SO₄, suggesting that adrenal P450c17 was an important source of prostatic androgen precursors.

3.2 P450c17 Inhibitors, Abiraterone, Orterenol, and Galeterone

Inhibitors of P450c17 have been developed to block the production of DHEA in the adrenal to prevent intracrine synthesis of T and DHT in prostate cancer. Abiraterone acetate (AA) is the lead agent that is FDA approved for this indication, Fig. 2 (Attard et al., 2008). Since AA blocks the formation of 17 α -hydroxypregnenolone it also inhibits the production of glucocorticoids. AA must therefore be coadministered with prednisone to prevent overstimulation of the adrenal by ACTH from the anterior pituitary. In the absence of adrenal-pituitary axis blockade, pregnenolone will be converted to progesterone and diverted to desoxycorticosterone, a potent mineralocorticoid, leading to mineralocorticoid excess and hypertension (Attard et al., 2012). Clinical trials in which CRPC patients that receive AA plus prednisone show an increase in progression free survival. This outcome is seen in CRPC patients, (Attard et al., 2009) and in metastatic CRPC patients that are either chemotherapy naïve (Fizazi et al., 2012) or have been treated with docetaxel (Danila et al., 2010; Reid et al., 2010).

AA is metabolized to 4 α -abiraterone by HSD3B1 where it represents (4% of the total metabolites). 4 α -Abiraterone has almost the same IC₅₀ value as abiraterone for P450c17, but also acts as an inhibitor of HSD3B1 and as an inhibitor of SRD5A (Li et al., 2015). 4-

Abiraterone also acts as an antagonist of AR and the mutated AR, suggesting that 4α -abiraterone may have a superior drug profile to AA (Li et al., 2015). However, 4α -abiraterone is reduced by SRD5A to the corresponding 5α -reduced metabolite which acts as an AR agonist (Li et al., 2016).

Second-generation inhibitors have been developed for P450c17 with the goal of preferentially inhibiting the 17,20-lyase activity to block DHEA production while sparing the 17α -hydroxylase activity. These inhibitors are desirable since they would permit the synthesis of cortisol in the adrenal which would negate the need to coadminister prednisone. For example, Orteronel (a nonsteroidal inhibitor of P450c17) shows preferential inhibition of the 17,20-lyase activity ($IC_{50}=140nM$) over the 17α -hydroxylase activity ($IC_{50}=760nM$) (Yamaoka et al., 2012), Fig. 2. However, clinical trials showed that Orteronel had no clinical benefit in terms of survival when the treatment arms of Orteronel plus prednisone vs prednisone alone were compared (Fizazi et al., 2015). As a result the further development of Orteronel has been abandoned.

Galeterone (TOK-001 or VN/124-1) was also developed to act as a P450c17 inhibitor, (Njar & Brodie, 2015) and showed a superior multifunctional activity profile to AA (Alyamani et al., 2017). Galeterone acts as a direct AR-antagonist on the wild-type AR and mutated AR and prevents the binding of the liganded receptor to chromatin in a manner similar to that reported for enzalutamide (ENZ). The drug was also found to be a selective AR degrader of the mutant AR (T878A) but not of the wild-type AR (Yu et al., 2014). Interestingly, Galeterone was also found to enhance degradation of the AR-V7 splice variant (Kwegyir-Afful, Ramalingam, Purushottamachar, Ramamurthy, & Njar, 2015). AR-V7 lacks the ligand-binding domain of the AR and is constitutively active in CRPC patients that become resistant to AA (Cao et al., 2014; Mostaghel et al., 2011). However, in the ARMOR3-SV trial this multifunctional activity was not found to be superior to the AR antagonist ENZ alone and the clinical development of Galeterone has been halted (Highlights, 2016).

Galeterone is also metabolized to 4α -galeterone and suppresses steroidogenesis, AR stability, and AR target gene expression with similar potency to the parent compound. But further metabolism by SRD5A limits these desirable properties (Alyamani et al., 2017). Thus, both Abiraterone and Galeterone are metabolized to 4α -3-ketosteroids that can enhance the properties of the parent drug. This has led to the concept that the superior profile of the 4α -3-ketosteroid metabolites could be exploited if either AA or Galeterone were coadministered with dutasteride to prevent metabolism to their 5α -reduced metabolites but this benefit has not yet been realized in clinical trials (McKay et al., 2017).

Crystal structures of P450c17 with both Abiraterone and Galeterone show that the ring nitrogen of the pyridine or benzimidazole ring at C17 in these respective drugs coordinates with the heme iron (DeVore & Scott, 2012). While the α -face of the steroid is enclosed by the active site, the active site is not complementary to the β -face of the steroid. The largest extension of the active site exists where the 3β -hydroxy group binds. The overall binding mode for both inhibitors suggests similarity in either the binding of the 3β -hydroxyl group or the binding of the 3-keto group present in 4α -abiraterone and 4α -galeterone similar to that

observed in binding steroids to the AR, and predicts that these metabolites will act as ligands for AR.

3.3 Prednisone Treatment

Prednisone is coadministered with P450c17 inhibitors to block the adrenal–pituitary axis to prevent the overstimulation of the adrenal and prevent mineralocorticoid excess. As prednisone by itself would block the production of DHEA, it is reasonable to ask whether there is any benefit to adding a P450c17 inhibitor to a regimen of prednisone alone, and whether a P450c17 inhibitor can be given in the absence of prednisone clinically. In clinical trials with AA and prednisone vs prednisone alone, there was a benefit from the drug combination (Ryan et al., 2015) but this was not seen when Orterenol when used in place of AA (see earlier) (Fizazi et al., 2015). Administration of AA alone resulted in an increase in mineralocorticoid production upstream from P450c17 as expected leading to hypertension, hypokalemia, and fluid overload. However, these symptoms could be reversed by the administration of either an antimineralocorticoid or dexamethasone (Attard et al., 2012). The requirement for coadministration of glucocorticoids could have a dark side in that as prostate cancer progresses mutations in the AR occur to make it ligand promiscuous so that the AR will use glucocorticoids as substitute ligands (Zhao et al., 2000). In addition, AR and GR share the same response element in the promoter regions of responsive genes so that the liganded-GR may substitute for the liganded-AR under conditions of androgen deprivation (Arora et al., 2013).

3.4 Drug Resistance Mechanisms

CRPC patients on P450c17 inhibitors invariably develop drug resistance (Buttiglierio et al., 2015). Resistance mechanisms can be multifaceted. These include: (i) incomplete inhibition of P450c17 (Tamae et al., 2015; Taplin et al., 2014); (ii) increased expression of steroidogenic enzymes in the tumor to exploit residual DHEA (Liu et al., 2017; Mostaghel et al., 2011); (iii) the presence of allelic variants in *HSD3BI* that increase enzyme stability (Chang et al., 2013); (iv) use of the backdoor pathway to DHT to by-pass DHEA (Auchus, 2004; Mohler, Titus, Bai, et al., 2011; Mohler, Titus, & Wilson, 2011); (v) AR gene amplification (Visakorpi et al., 1995); (vi) AR mutations (Taplin et al., 1995); and (vii) emergence of AR-SV's (Cao et al., 2014; Mostaghel et al., 2011). These mechanisms are not mutually exclusive. Some of these mechanisms could be surmounted by the use of an AR receptor antagonist, e.g., ENZ (Tran et al., 2009). Use of combination therapy of AA and ENZ has met with mixed success. Some clinical benefit is associated with the sequential use of AA followed by ENZ but the reverse does not hold (Miyake et al., 2017). Interrogation of the mechanisms of drug resistance can be improved by the sensitive and accurate quantitation of serum and prostatic androgens.

4. ANALYTICAL CHEMISTRY TO MEASURE KETO-AND HYDROXYANDROGENS

Traditional methods of measuring androgens have used radioimmunoassays which are unreliable for the following reasons: (i) they can only measure one analyte at a time and multiple different antibodies have to be used of different specificity and sensitivity; (ii) there

is no structural identity of the analyte being measured; and (iii) it is not possible to control for unknowns in the biospecimens that may react with the antibody leading to false positives (Penning, Lee, Jin, Gutierrez, & Blair, 2010).

The gold-standard is to use stable-isotope dilution liquid chromatography tandem mass spectrometry (SID-LC-MS/MS). This method separates out the analytes by HPLC and identifies the analyte based on three criteria: retention time, exact mass, and precursor-product ion transitions. The method uses either ^{13}C or ^2H internal standards that only differ from the analyte being measured in mass due to the presence of the heavy isotope. Thus, exact quantitation can then be interpolated by the ratio method (Penning et al., 2010).

Androgens do pose a challenge to be measured by this technology. First, androgens are present as a number of regio- or stereoisomers that will have the same exact mass, e.g., DHEA, T, and epi-T are all isomers; and similarly, DHT, androsterone, and epi-androsterone are all isomers. Thus, chromatographic separation of these analytes is essential. Second, androgens have poor ionization properties and thus derivatization with preionization derivatives is essential to attain the desired sensitivity (Penning et al., 2010). Third, androgens may contain only keto- or hydroxy groups and therefore two derivatization procedures are required to measure a complete androgen profile. We have used Girard-T oximes to derivatize ketoandrogens, where the charged trimethylamine group gives a strong positive ion and mass transition to follow (Tamae et al., 2013). We have also used picolinic acid to derivatize hydroxyandrogens, where the charged picolinate ester gives a strong positive ion and mass transition (Zang, Tamae, et al., 2017). Using these methods, nine androgens plus their glucuronide and sulfate conjugates can be quantified with precision and accuracy in the low or sub-pg range. Detection of the glucuronide or sulfate conjugates requires enzymatic hydrolysis with β -glucuronidase and aryl sulfatase, respectively. These methods have been used in a number of clinical trials: (i) the TAPS trial (Mostaghel et al., 2014) and (ii) the neoadjuvant Leuprolide plus AA clinical trial (Taplin et al., 2014).

5. EVIDENCE FOR A DEPOT OF DHEA-SO₄

5.1 DHEA-SO₄

Using SID-LC-MS/MS methods, we examined the efficacy of a neoadjuvant clinical trial using Leuprolide and AA to reduce serum androgens (Taplin et al., 2014). This trial had two groups. In group 1, patients received Leuprolide for 12 weeks and then for a subsequent 12 weeks they received Leuprolide, AA, and prednisone. In group 2, patients received Leuprolide, AA, and prednisone for all 24 weeks. Serum androgen levels were measured at base-line, at 12 and 24 weeks for all patients. Group 1 allowed us to compare the efficacy of Leuprolide alone vs the combined drug treatment. Data showed that after 12 weeks of Leuprolide alone testicular androgens, e.g., T and DHT fell to castrate levels, whereas DHEA-SO₄ and DHEA were unaffected. Subsequent administration of AA caused no further change in T and DHT but now DHEA-SO₄ and DHEA were reduced by 90%. However, since DHEA-SO₄ is present in μM concentrations, the amount that remains after Leuprolide, AA plus prednisone is 4000 times higher than the castrate levels of circulating T achieved. This suggests that despite best efforts to inhibit P450c17 a substantial reservoir of DHEA-SO₄ remains to fuel intraprostatic and androgen biosynthesis. It was predicted that

all inhibitors that target P450c17 would have the same property and that this will lead to their therapeutic failure (Tamae et al., 2015). In several clinical trials with AA, the lower limit of quantitation for DHEA-SO₄ was 15µg/dL which is 2000 times higher than castrate levels of T and the presence of this reservoir may not have been appreciated, (Attard et al., 2008; Ryan et al., 2010).

5.2 5-Androstenediol-SO₄ and Epi-Androsterone-SO₄ as a Source of Intratumoral Androgens

We subsequently applied our SID-LC-MS/MS method for hydro-xyandrogen measurement to patient samples from the neoadjuvant Leuprolide/AA clinical trial (Zang, Taplin, et al., 2017). This enabled us to measure diols not previously measured, e.g., 5-androstene-3β,17β-diol (5-Adiol), 5α-androstane-3α,17β-diol (3α-diol), and 5α-androstane-3β,17-β-diol (3β-diol) in the serum samples. We reasoned that the design of the trial would permit us for the first time to discriminate between testicular and adrenal sources of androgens in prostate cancer patients. Those androgens that were reduced significantly after Leuprolide treatment would be of testicular origin, while those androgens that were only reduced after the addition of AA plus prednisone would be of adrenal origin. We find that T, DHT, 3α-adiol, and 3β-adiol were predominately of testicular origin. By contrast, DHEA, epi-androsterone and their conjugates, 5-Adiol-SO₄, and 5-Adiol glucuronide were predominately of adrenal origin. Our findings also show that ADT failed to completely suppress DHEA-SO₄ levels and that two unappreciated sources of intratumoral androgens that were not suppressed by Leuprolide acetate alone were 5-Adiol-SO₄ and epiandrosterone-SO₄. Pathways by which 5-Adiol-SO₄ and epi-androsterone-SO₄ could be converted to T and DHT are shown in Fig. 3.

5.3 Steroid Sulfate Transport

Steroid sulfates that could act as depots for intratumoral androgen biosynthesis in prostate cancer include DHEA-SO₄, 5-Adiol-SO₄, and epi-androsterone-SO₄. *SLCO*-encoded organic anion transporting polypeptides (OATP) will dictate whether these steroids can be taken up by prostate tumors (Arakawa et al., 2012; Green et al., 2017). Microarray measurements of *SLCO* transcripts in LNCaP and 22Rv1 cells grown under conditions of androgen depletion to mimic ADT demonstrated that *SLCO1A2* mRNA expression was markedly upregulated (4-fold), and to a lesser extent *SLCO1B1* (2.5-fold) and *SLCO1B2* (1.74-fold) were increased in expression as well. These changes were confirmed by qRT-PCR by monitoring increases in expression at 3 and 7 days postandrogen depletion. Increased expression was accompanied by increased cellular uptake of [³H]-DHEA-SO₄ and increased cellular proliferation stimulated by DHEA-SO₄. Cellular proliferation in response to DHEA-SO₄ was attenuated by *SLCO1A2* knockdown. *SLCO1A2* was also downregulated by DHT, suggesting that its expression is repressed by androgens (Arakawa et al., 2012).

SLCO1B1- and *SLCO2B1*-expressing prostate cancer xenografts showed a 3.9- and 1.9-fold increase in tumor accumulation of DHEA-SO₄, respectively, and a 1.6- and 2.7-fold increase in DHEA, respectively (Green et al., 2017). The same genes were found to be overexpressed in CRPC metastases and were associated with prostate cancer mortality when 1309 Caucasian prostate cancer patients were genotyped (Wright et al., 2011). 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 (TTP) on ADT ($P<0.05$). The differences in median TTP for each of these polymorphisms were about 10 months (Yang et al., 2012). In LNCaP cells transfected with the *SLCO2B1* SNP variant rs1242149 (935G>A; Arg312Gln), the variant exhibited higher maximal DHEA-SO₄ uptake when compared with the wild-type allele (Yang et al., 2012). This *SLCO2B1* genotype, which allows more efficient import of androgen and enhances cell growth (Yang et al., 2012). Collectively, these findings indicate that changes in expression of *SLCO* transporters and genotype can determine response to ADT, and that they increase the availability of DHEA-SO₄ for intracrine androgen biosynthesis.

5.4 Steroid Sulfatase

DHEA-SO₄ and other sulfates that are transported into prostate tumors must be hydrolyzed by STS to liberate the free steroid for downstream steroidogenesis, (Klein, Molwitz, & Bartsch, 1989), Fig. 3. Only one STS is known to be expressed in humans (Reed, Purohit, Woo, Newman, & Potter, 2005; Yen et al., 1987). The crystal structure of human STS identified a formylglycine residue at the active site that can be hydrated to form a hydroxyl-formylglycine (gem-diol) which is sulfonated during the hydrolysis of sulfonated steroids (Gosh, 2007). The sulfonated enzyme intermediate then hydrolyzes to liberate HSO₄⁻ and regenerate the formylglycine residue.

STS is regulated by six different tissue-specific promoters, however, there have been few studies on STS expression by tissue-specific promoters in human prostate. Nevertheless, STS has been detected in prostate tissue where the highest activity is in the prostatic epithelium (Farnsworth, 1973; Klein et al., 1989); and it is also expressed in LNCaP cells (Dalla Valle et al., 2007; Selcer, Kabler, Sarap, Xiao, & Li, 2002). The pivotal role of STS in the liberation of free DHEA for intracrine androgen synthesis has prompted the development of both irreversible steroidal- and nonsteroidal-based STS inhibitors, STX213 (Fischer, Chander, et al., 2003; Fischer, Woo, et al., 2003) and STX64, respectively (Woo, Purohit, Malini, Reed, & Potter, 2000). These compounds each contain a sulfamate group which can inactivate STS by one of two mechanisms. The sulfamate can either attack the formylglycine residue or a nucleophile on the enzyme can attack the sulfamate to produce a sulfamoylated enzyme. In either case, irreversible enzyme inhibition is achieved, Fig. 4. These compounds have yet to be used in clinical trials of CRPC. It is noteworthy that X-linked ichthyosis is characterized by a loss of STS, raising concerns about the possible long-term adverse side effects of using sulfatase inhibitors for prostate cancer (Alperin & Shapiro, 1997; Webster, France, Shapiro, & Weiss, 1978).

6. EVIDENCE FOR DE NOVO SYNTHESIS OF T AND DHT FROM CHOLESTEROL OR PREGNENOLONE IN CRPC TUMORS

The large amount of DHEA-SO₄ that remains in the serum even after P450c17 inhibition raises the issue as to whether prostate cancer cells need to synthesize androgens from cholesterol when there is such a significant amount of downstream precursor available. Patient-derived xenografts that are AA resistant show a 1.72-fold increase in *CYP17A1*

transcript and still synthesize a basal level of T and DHT (Mostaghel et al., 2011). Measurement of [^{14}C]-acetate conversion into androgens in LNCaP cells showed that whereas there was conversion into a lipid fraction containing cholesterol, there was only minor conversion into progesterone, 17 α -hydroxyprogesterone, and DHT (Locke et al., 2008). When CRPC tumors isolated from castrate mice were incubated ex vivo with [^{14}C]-acetic acid, formation of progesterone (1.5%); 17 α -hydroxyprogesterone (4.0%); and DHT (8.3%) based on radiochromatography was observed (Locke et al., 2008). Because other analytes might coelute with DHT and no MS data were presented to support analyte identity these findings are equivocal at best. MS data were provided to support the formation of 5 α -dihydroprogesterone, but without evidence for a functional P450c17 reaction it is uncertain whether these tumor specimens can really convert acetic acid to DHT. In a follow-up study, LNCaP cells were incubated with [^3H]-progesterone as precursor. While significant amounts were converted into 5 α -pregnane-3,17-diol-20-one (a precursor in the backdoor pathway to DHT) less than 0.2% and 0.04% was converted to T and DHT, respectively (Locke et al., 2009). Collectively, these data do not strongly support de novo steroidogenesis nor do they support the conversion of C21 steroids into C19 steroids in CRPC.

7. ROLE OF HSD3B1 IN INTRATUMORAL ANDROGEN BIOSYNTHESIS

HSD3B1 is the isoform that converts DHEA to 4 -AD, and 5-Adiol to T in the prostate. The enzyme is bifunctional in that it catalyzes the NAD^+ -dependent dehydrogenation of the 3 β -hydroxy group of a 5 -ene and then catalyzes the isomerization of the resultant 5 -3-ketosteroid to yield the 4 -3-ketosteroid. In the case of epi-androsterone only the dehydrogenation step is required to produce 5-Adione. A common allelic *HSD3B1*(1245C) exists in approximately 22% of the Caucasian population but varies widely by ethnicity. The mutation N367T does not affect the steady-state kinetic constants for the enzyme but increases enzyme stability by preventing its proteasomal degradation (Chang et al., 2013). This stability mutant will increase the flux to DHEA down the steroidogenic pathways to DHT and could contribute to AA drug resistance. The same mutant would, however, increase formation of 4 -abiraterone and 4 -galeterone which would be advantageous based on their superior properties. The differential effects of this mutation on promoting steroidogenesis and improving the P450c17 inhibitor profile remain to be resolved.

8. ROLE OF AKR1C3 IN INTRATUMORAL ANDROGEN BIOSYNTHESIS

One of the most overexpressed genes in CRPC is *AKR1C3*, type 5 17 β -hydroxysteroid dehydrogenase. This is seen in prostate cancer cells grown in androgen-depleted media (Hofland et al., 2010), in xenografts in castrate mice (Hofland et al., 2010; Liu et al., 2017, 2015), in patient tumor samples, and in soft-tissue metastases (Stanbrough et al., 2006). Nine clinical studies have reported similar findings for AKR1C3 expression in biospecimens from human patients (Fankhauser et al., 2014; Hamid et al., 2012; Hofland et al., 2010; Jernberg et al., 2013; Mitsiades et al., 2012; Pfeiffer, Smit, Sedelaar, & Schalken, 2011; Powell et al., 2015; Stanbrough et al., 2006; Tian et al., 2014). AKR1C3 plays a pivotal role in all pathways to DHT. It catalyzes the conversion of 4 -AD to T, the conversion of 5-Adione to DHT, and the conversion of DHEA to 5-Adiol. It is also involved in the backdoor pathway where it converts androsterone to 3 α -diol (Byrns, Mindnich, Duan, & Penning,

2012; Lin et al., 1997; Penning et al., 2000). AKR1C3 expression is upregulated by ADT and repressed by androgens and its overexpression is part of the resistance mechanism to AA and ENZ (Liu et al., 2017, 2015). AKR1C3 expression is driven by the TMPRSS2-ERG fusion protein by binding to the *AKR1C3* promoter (Powell et al., 2015). A feed-forward mechanism has been proposed in which TMPRSS2-ERG expression increases as the tumor begins to synthesize more T and DHT and this displaces the AR from the *AKR1C3* promoter to enhance intratumoral androgen biosynthesis (Powell et al., 2015).

We have proposed that the residual DHEA-SO₄ that exists after P450c17 inhibition coupled with AKR1C3 overexpression creates a “perfect-storm” for AA drug resistance (Tamae et al., 2015). In mouse xenografts of CWR22Rv1 prostate tumors that are resistant to AA and ENZ, drug resistance can be surmounted by indomethacin (Liu et al., 2017, 2015), a selective and potent inhibitor of AKR1C3 identified by us (Byrns, Steckelbroeck & Penning, 2008). AKR1C3 inhibitors thus have a place in CRPC treatment especially in patients who progress on second-line ADT.

There are a number of nonsteroidal- (Adeniji et al., 2012; Kikuchi et al., 2014; Liedtke et al., 2013; Yepuru et al., 2013; Yin et al., 2014) and steroidal-based inhibitors of AKR1C3 (Bothe, Cancho-Grande, Irlbach, & Ray, 2015) in preclinical development. Several are deserving of mention: SN33638, a sulfonyl piperidine was found to be only effective in high-AKR1C3 expressing prostate cancer cells, e.g., LAPC4 or 22Rv1 (Yin et al., 2014). ASP9521 developed by Astellas with desirable preclinical properties for the inhibition of AKR1C3 was taken into a phase I/IIb clinical trial where it was well tolerated but without efficacy in 7/13 patients (Kikuchi et al., 2014; Lortol et al., 2014). The authors concluded that the lack of drug efficacy may have been due to the fact that individuals were excluded who had been recently on ADT and that therefore AKR1C3 was not expressed in sufficient amounts to be targeted (Lortol et al., 2014). Collectively, these data suggest that when AKR1C3 is overexpressed as part of the adaptive response to ADT a therapeutic benefit of AKR1C3 inhibition is anticipated.

9. CONCLUSIONS AND FUTURE DIRECTIONS

DHEA-SO₄ is present at 400–1000 times the concentration of DHEA and is of adrenal origin. Inhibition of P450c17, the primary source of DHEA, leaves a sizable pool of DHEA-SO₄ available for intratumoral bio-synthesis of T and DHT in CRPC. As CRPC progresses the tumor can stay dependent upon androgens due to adaptive responses that can exploit this residual pool of DHEA-SO₄. These adaptive responses include increased expression of *SLCO* transporters and *AKR1C3*. Whether changes in *STS* expression coincide with these adaptive responses remains to be determined. STS inhibitors and AKR1C3 inhibitors could be used to block steroidogenesis downstream from P450c17, they could be used to surmount P450c17 inhibitor drug resistance and could be used as monotherapies.

ACKNOWLEDGMENTS

This work is supported by Grants P01-CA163227 and P30-ES013508 from the National Cancer Institute and the National Institute of Environmental Health Sciences, respectively.

REFERENCES

- Adeniji AO, Twenter BM, Byrns MC, Jin Y, Chen M, Winkler JD, et al. (2012). 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. *Journal of Medicinal Chemistry*, 55, 2311–2323. [PubMed: 22263837]
- Alperin ES, & Shapiro LJ (1997). Characterization of point mutations in patients with X-linked ichthyosis. Effects on the structure and function of the steroid sulfatase protein. *Journal of Biological Chemistry*, 272, 20746–20763.
- Alyamani M, Li Z, Berk M, Li J, Tang J, Upadhyay S, et al. (2017). Steroidogenic metabolism of galeterone reveals a diversity of biochemical activities. *Cell Chemical Biology*, 24, 825–832. [PubMed: 28648378]
- Andriole GL, Humphrey P, Ray P, Gleave ME, Trachtenberg J, Thomas LN, et al. (2004). Effect of the dual 5 α -reductase inhibitor dutasteride on markers of tumor regression in prostate cancer. *Journal of Urology*, 172, 915–919. [PubMed: 15310997]
- Arakawa H, Nakanishi T, Yanagihara C, Nishimoto T, Wakayama T, Mizokami A, et al. (2012). 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. *Biochemical Pharmacology*, 84, 1070–1077. [PubMed: 22864060]
- Arora VK, Schenkein E, Murali R, Subudhi SK, Wongvipat J, Balbas MD, et al. (2013). Glucocorticoid receptor confers resistance to antiandrogens by bypassing androgen receptor blockade. *Cell*, 155, 1309–1322. [PubMed: 24315100]
- Attard G, Reid AH, A'Hern R, Parker C, Oommen NB, Folkard E, et al. (2009). Selective inhibition of CYP17 with abiraterone acetate is highly active in the treatment of castration-resistant prostate cancer. *Journal of Clinical Oncology*, 27, 3742–3748. [PubMed: 19470933]
- Attard G, Reid AH, Auchus RJ, Hughes BA, Cassidy AM, Thompson E, et al. (2012). Clinical and biochemical consequences of CYP17A1 inhibition with abiraterone given with and without exogenous glucocorticoids in castrate men with advanced prostate cancer. *The Journal of Clinical Endocrinology and Metabolism*, 97, 507–516. [PubMed: 22170708]
- Attard G, Reid AH, Yap TA, Raynaud F, Dowsett M, Setttee S, et al. (2008). Phase 1 clinical trial of a selective inhibitor of CYP17, abiraterone acetate, confirms that castration-resistant prostate cancer commonly remains hormone driven. *Journal of Clinical Oncology*, 28, 4563–4571.
- Auchus RJ (2004). The backdoor pathway to dihydrotestosterone. *Trends in Endocrinology and Metabolism*, 15, 432–438. [PubMed: 15519890]
- Auchus RJ, Lee TC, & Miller WL (1998). Cytochrome b5 augments the 17,20-lyase activity of human P450c17 without direct electron transfer. *Journal of Biological Chemistry*, 273, 3158–3165. [PubMed: 9452426]
- Bothe U, Cancho-Grande Y, Irlbach ER, & Ray NC (2015). 3-Nitrogen or sulphur substituted oestra-1,3,5(10),16-tetraene AKR1C3 inhibitors world intellectual property organization WO 2016037956.
- Buttiglieri C, Tucci M, Bertaglia V, Vignani F, Bironzo P, Di Maio M, et al. (2015). Understanding and overcoming the mechanisms of primary and acquired resistance to abiraterone and enzalutamide in castration resistant prostate cancer. *Cancer Treatment Reviews*, 41, 884–892. [PubMed: 26342718]
- Byrns MC, Mindnich R, Duan L, & Penning TM (2012). Overexpression of aldoketo reductase 1C3 (AKR1C3) in LNCaP cells diverts androgen metabolism towards testosterone resulting in resistance to the 5 α -reductase inhibitor finasteride. *The Journal of Steroid Biochemistry and Molecular Biology*, 130, 7–15. [PubMed: 22265960]
- Byrns MC, Steckelbroeck S, & Penning TM (2008). 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. *Biochemical Pharmacology*, 75, 484–493. [PubMed: 17950253]

- Cao B, Qi Y, Zhang G, Xu D, Zhan Y, Alvarez X, et al. (2014). Androgen receptor splice variants activating the full-length receptor in mediating resistance to androgen-directed therapy. *Oncotarget*, 5, 1646–1656. [PubMed: 24722067]
- Chang K-H, Li R, Kuri B, Lotan Y, Roehrborn CG, Liu J, et al. (2013). A gain-of-function mutation in DHT synthesis in castration-resistant prostate cancer. *Cell*, 154, 1074–1084. [PubMed: 23993097]
- Chang KH, Li R, Papari-Zareei M, Watumull L, Zhao YD, Auchus RJ, et al. (2011). Dihydrotestosterone synthesis bypasses testosterone to drive castration-resistant prostate cancer. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 13728–13733. [PubMed: 21795608]
- Cho E, Montgomery RB, & Mostaghel EA (2014). Minireview: SLCO and ABC transporters: A role for steroid transport in prostate cancer progression. *Endocrinology*, 155, 4124–4132. [PubMed: 25147980]
- Chung BC, Picado-Leonard J, Haniu M, Bienkowski M, Hall PF, Shively JE, et al. (1987). Cytochrome P450c17 (steroid 17 alpha-hydroxylase/17,20 lyase): Cloning of human adrenal and testis cDNAs indicates the same gene is expressed in both tissues. *Proceedings of the National Academy of Sciences of the United States of America*, 84, 407–411. [PubMed: 3025870]
- Dalla Valle L, Toffolo V, Nardi A, Fiore C, Armanini D, Belvedere P, et al. (2007). The expression of the human steroid sulfatase-encoding gene is driven by alternative first exons. *The Journal of Steroid Biochemistry and Molecular Biology*, 107, 22–29. [PubMed: 17601726]
- Damgaard-Olesen A, Johannsen TH, Holmboe SA, Sjøberg T, Petersen JH, Andersson A, et al. (2016). Reference ranges of 17-hydroxyprogesterone, DHEA, DHEAS, androstenedione, total and free testosterone determined by TurboFlow-LCMS/MS and associations to health markers in 304 men. *Clinical Chimica Acta*, 454, 82–88.
- Danila DC, Morris MJ, de Bono JS, Ryan CJ, Denmeade SR, Smith MR, et al. (2010). Phase II multicenter study of abiraterone acetate plus prednisone therapy in patients with docetaxel-treated castration-resistant prostate cancer. *Journal of Clinical Oncology*, 28, 1496–1501. [PubMed: 20159814]
- DeVore NM, & Scott EE (2012). Structures of cytochrome P450 17A1 with prostate cancer drugs abiraterone and TOK-001. *Nature*, 482, 116–119. [PubMed: 22266943]
- Estabrook RW, Shet MS, Fisher CW, Jenkins CM, & Waterman MR (1996). The interaction of NADPH-P450 reductase with P450: An electrochemical study of the role of the flavin mononucleotide-binding domain. *Archives of Biochemistry and Biophysics*, 333, 308–315. [PubMed: 8806785]
- Fankhauser M, Tan Y, Macintyre G, Haviv I, Hong MKH, Nguyen A, et al. (2014). Canonical androstenedione reduction is the predominant source of signaling androgens in hormone-refractory prostate cancer. *Clinical Cancer Research*, 20, 5547–5557. [PubMed: 24771644]
- Farnsworth WE (1973). Human prostatic dehydroepiandrosterone sulfate sulfatase. *Steroids*, 21, 647–664. [PubMed: 4267483]
- Fischer DS, Chander SK, Woo LW, Fenton JC, Purohit A, Reed MJ, et al. (2003). Novel D-ring modified steroid derivatives as potent, non-estrogenic, steroid sulfatase inhibitors with in vivo activity. *The Journal of Steroid Biochemistry and Molecular Biology*, 84, 343–349. [PubMed: 12711021]
- Fischer DS, Woo LWL, Mahon MF, Purohit A, Reed MJ, & Potter BVL (2003). D-ring modified estrone derivatives as novel potent inhibitors of steroid sulfatase. *Bioorganic & Medicinal Chemistry*, 11, 1685–1700. [PubMed: 12659755]
- Fizazi K, Jones R, Oudard S, Efsthathiou E, Saad F, de Wit R, et al. (2015). Phase III, randomized, double-blind, multicenter trial comparing orteronel (TAK-700) plus prednisone with placebo plus prednisone in patients with metastatic castration-resistant prostate cancer that has progressed during or after docetaxel-based therapy: ELM-PC 5. *Journal of Clinical Oncology*, 33, 723–731. [PubMed: 25624429]
- Fizazi K, Scher HI, Molina A, Logothetis CJ, Chi KN, Jones RJ, et al. (2012). 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 Oncology*, 983–992. [PubMed: 22995653]

- Furr BJ, Valcaccia B, Curry B, Woodburn JR, Chesterson G, & Tucker H (1987). ICI 176,334: A novel non-steroidal, peripherally selective antiandrogen. *Journal of Endocrinology*, 113, R7–R9. [PubMed: 3625091]
- Gosh D (2007). Human sulfatases: A structural perspective to catalysis. *Cellular and Molecular Life Sciences*, 64, 2013–2022.
- Green SM, Kaipainen A, Bullock K, Zhang A, Lucas JM, Matson C, et al. (2017). Role of OATP transporters in steroid uptake by prostate cancer cells in vivo. *Prostate Cancer and Prostatic Diseases*, 20, 20–27. [PubMed: 27645128]
- Hamid AR, Pfeiffer MJ, Verhaegh GW, Schaafsma E, Brandt A, Sweep FC, et al. (2012). Aldo-keto reductase family 1 member C3 (AKR1C3) is a biomarker and therapeutic target for castration-resistant prostate cancer. *Molecular Medicine*, 18, 1449–1455.
- Highlights GN (2016). Tokai ends phase III trial of lead candidate galeterone. *Genetic Engineering & Biotechnology News* <https://www.genengnews.com/gen-news-highlights/tokai-ends-phase-iii-trial-of-lead-candidate-galeterone/81253001> [Press release; July 26, 2016].
- Hofland J, van Weerden WM, Dits NFJ, Steenbergen J, van Leenders GH, Jenster G, et al. (2010). Evidence of limited contributions for intratumoral steroidogenesis in prostate cancer. *Cancer Research*, 70, 1256–1264. [PubMed: 20086173]
- Huggins CB (1965). Two principles in endocrine therapy of cancers: Hormone deprivation and hormone interference. *Cancer Research*, 25, 1163–1167. [PubMed: 5897831]
- Huggins C, Stevens R, & Hodges CV (1941). Studies on prostatic cancer: II. The effects of castration on advanced carcinoma of the prostate gland. *Archives of Surgery*, 43, 209–223.
- Jernberg E, Thysell E, Ylitalo EB, Rudolfsson S, Crnalic S, Widmark A, et al. (2013). Characterization of the prostate cancer bone metastases according to expression levels of steroidogenic enzymes and androgen receptor splice variants. *PLoS One*, 8, e77407. [PubMed: 24244276]
- Kikuchi A, Furutani T, Azami H, Watanabe K, Nimi T, Kamiyama Y, et al. (2014). 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). *Investigational New Drugs*, 32, 860–870. [PubMed: 24981575]
- Klein H, Molwitz T, & Bartsch W (1989). Steroid sulfate sulfatase in human benign prostatic hyperplasia: Characterization and quantification of the enzyme in epithelium and stroma. *Journal of Steroid Biochemistry*, 33, 195–200. [PubMed: 2475673]
- Knudsen K, & Penning TM (2010). Partners in crime: Deregulation of AR activity and androgen synthesis in prostate cancer. *Trends in Endocrinology and Metabolism*, 21, 315–324. [PubMed: 20138542]
- Knudsen K, & Scher HI (2009). Starving the addiction: New opportunities for durable suppression of AR signaling in prostate cancer. *Clinical Cancer Research*, 15, 4792–4798. [PubMed: 19638458]
- Kühn-Velten WN, & Lessmann M (1992). Ketoconazole inhibition of the bifunctional cytochrome P450c17 does not affect androgen formation from the endogenous lyase substrate. The catalytic site remains refractory in the course of intermediary hydroxyprogesterone processing. *Biochemical Pharmacology*, 44, 2371–2378. [PubMed: 1472102]
- Kwegyir-Afful AK, Ramalingam S, Purushottamachar P, Ramamurthy VP, & Njar VC (2015). Galeterone and VNPT55 induce proteasomal degradation of AR/AR-V7, induce significant apoptosis via cytochrome c release and suppress growth of castration resistant prostate cancer xenografts in vivo. *Oncotarget*, 6, 27440–27460. [PubMed: 26196320]
- Labrie F, Belanger A, Luu-The V, Labrie C, Simard J, Cusan L, et al. (1998). DHEA and the intracrine formation of androgens and estrogens in peripheral target tissues: Its role during aging. *Steroids*, 63, 322–328. [PubMed: 9618795]
- Labrie F, Bélanger A, Simard J, Luu-The V, & Labrie C (1995). DHEA and peripheral androgen and estrogen formation: Intracrinology. *Annals of the New York Academy of Sciences*, 774, 16–28. [PubMed: 8597456]
- Li Z, Alyamani M, Li J, Rogacki K, Abazeed M, Upadhyay SK, et al. (2016). Redirecting abiraterone metabolism to fine-tune prostate cancer anti-androgen therapy. *Nature*, 533, 547–551. [PubMed: 27225130]

- Li Z, Bishop AC, Alyamani M, Garcia JA, Dreicer R, Bunch D, et al. (2015). Conversion of abiraterone to D4A drives anti-tumour activity in prostate cancer. *Nature*, 523, 347–351. [PubMed: 26030522]
- Liedtke AJ, Adeniji AO, Chen M, Byrns MC, Jin Y, Christianson DW, et al. (2013). 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. *Journal of Medicinal Chemistry*, 56, 2429–2446. [PubMed: 23432095]
- Lin H-K, Jez JM, Schlegel BP, Peehl DM, Pachter JA, & Penning TM (1997). Expression and characterization of recombinant type 2 3 α -hydroxysteroid dehydrogenase (HSD) from human prostate: Demonstration of bifunctional 3 α /17 β -HSD activity and cellular distribution. *Molecular Endocrinology*, 11, 1971–1984. [PubMed: 9415401]
- Liu C, Armstrong CM, Lou W, Lombard A, Evans CP, & Gao AC (2017). Inhibition of AKR1C3 activation overcomes resistance to abiraterone in advanced prostate cancer. *Molecular Cancer Therapeutics*, 16, 35–44. [PubMed: 27794047]
- Liu C, Lou W, Zhu Y, Yang JC, Nadiminty N, Gaikwad NW, et al. (2015). Intracrine androgens and AKR1C3 activation confer resistance to enzalutamide in prostate cancer. *Cancer Research*, 75, 1413–1422. [PubMed: 25649766]
- Locke JA, Guns ES, Lubik AA, Adomat HH, Hendy SC, Wood CA, et al. (2008). Androgen levels increase by intratumoral de novo steroidogenesis during progression of castration-resistant prostate cancer. *Cancer Research*, 68, 6407–6415. [PubMed: 18676866]
- Locke JA, Nelson CC, Adomat HH, Hendy SC, Gleave ME, & Guns ES (2009). Steroidogenesis inhibitors alter but do not eliminate androgen synthesis mechanisms during progression to castration-resistance in LNCaP prostate xenografts. *The Journal of Steroid Biochemistry and Molecular Biology*, 115, 126–136. [PubMed: 19442514]
- Loriot Y, Fizazi K, Jones RJ, Brand VDJ, Molife RL, Omlin A, et al. (2014). 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. *Investigational New Drugs*, 32, 995–1004. [PubMed: 24771350]
- McKay RR, Werner L, Mostaghel EA, Lis R, Voznesensky O, Zhang Z, et al. (2017). A phase II trial of abiraterone combined with dutasteride for men with metastatic castration-resistant prostate cancer. *Clinical Cancer Research*, 23, 935–945. [PubMed: 27683182]
- Mitsiades N, Sung CC, Schultz N, Danila DC, He B, Eedunuri VK, et al. (2012). Distinct patterns of dysregulated expression of enzymes involved in androgen synthesis and metabolism in metastatic prostate cancer tumors. *Cancer Research*, 72, 6142–6152. [PubMed: 22971343]
- Miyake H, Hara T, Tamura K, Sugiyama T, Furuse H, Ozono S, et al. (2017). Comparative assessment of efficacies between 2 alternative therapeutic sequences with novel androgen receptor-axis-targeted agents in patients with chemotherapy-naïve metastatic castration-resistant prostate cancer. *Clinical Genitourinary Cancer*, 15, e591–e597. [PubMed: 28063845]
- Mohler JL, Titus MA, Bai S, Kennerley BJ, Lih FB, Tomer KB, et al. (2011). Activation of the androgen receptor by intratumoral bioconversion of androstenediol to dihydrotestosterone in prostate cancer. *Cancer Research*, 71, 1486–1496. [PubMed: 21303972]
- Mohler JL, Titus MA, & Wilson EM (2011). Potential prostate cancer drug target: Bioactivation of androstenediol by conversion to dihydrotestosterone. *Clinical Cancer Research*, 17, 5844–5849. [PubMed: 21705451]
- Mostaghel EA, Marck BT, Plymate SR, Vessella RL, Balk S, Matsumoto AM, et al. (2011). Resistance to CYP17A1 inhibition with abiraterone in castrate-resistance prostate cancer: Induction of steroidogenesis and androgen receptor splice variants. *Clinical Cancer Research*, 17, 5913–5925. [PubMed: 21807635]
- Mostaghel EA, Nelson PS, Lange P, Lin DW, Taplin ME, Balk S, et al. (2014). Targeted androgen pathway suppression in localized prostate cancer: A pilot study. *Journal of Clinical Oncology*, 32, 229–237. [PubMed: 24323034]
- Nakajin S, Shinoda M, Haniu M, Shively JE, & Hall PF (1984). C21 steroid side chain cleavage enzyme from porcine adrenal microsomes. Purification and characterization of the 17 α -hydroxylase/C17,20-lyase cytochrome P-450. *Journal of Biological Chemistry*, 259, 3971–3976. [PubMed: 6608525]

- Njar VC, & Brodie AM (2015). Discovery and development of galeterone (TOK-001 or VN/124-1) for the treatment of all stages of prostate cancer. *Journal of Medicinal Chemistry*, 58, 2077–2087. [PubMed: 25591066]
- Peng HM, Im SC, Pearl NM, Turcu AF, Rege J, Waskell L, et al. (2016). Cytochrome b5 activates the 17,20-lyase activity of human cytochrome P450 17A1 by increasing the coupling of NADPH consumption to androgen production. *Biochemistry*, 55, 4356–4365. [PubMed: 27426448]
- Penning TM, Burczynski ME, Jez JM, Hung C-F, Lin H-K, Ma H, et al. (2000). Human 3 α -hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo keto reductase superfamily: Functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *Biochemical Journal*, 351, 67–77. [PubMed: 10998348]
- Penning TM, Lee SH, Jin Y, Gutierrez A, & Blair IA (2010). Liquid chromatography-mass spectrometry (LC-MS) of steroid hormone metabolites and its applications. *The Journal of Steroid Biochemistry and Molecular Biology*, 118, 177–187. [PubMed: 20036328]
- Pfeiffer MJ, Smit FP, Sedelaar JP, & Schalken JA (2011). Steroidogenic enzymes and stem cell markers are upregulated during androgen deprivation in prostate cancer. *Molecular Medicine*, 17, 657–664. [PubMed: 21365123]
- Powell K, Semaan L, Conley-LaComb MK, Asangani I, Wu YM, Ginsburg KB, et al. (2015). ERG/AKR1C3/AR constitutes a feed-forward loop for AR signaling in prostate cancer cells. *Clinical Cancer Research*, 21, 2569–2579. [PubMed: 25754347]
- Reed MJ, Purohit A, Woo LW, Newman SP, & Potter BV (2005). Steroid sulfatase: Molecular biology, regulation, and inhibition. *Endocrine Reviews*, 26, 171–202. [PubMed: 15561802]
- Reid AH, Attard G, Danila DC, Oommen NB, Olmos D, Fong PC, et al. (2010). Significant and sustained antitumor activity in post-docetaxel, castration-resistant prostate cancer with the CYP17 inhibitor abiraterone acetate. *Journal of Clinical Oncology*, 28, 1489–1495. [PubMed: 20159823]
- Ryan CJ, Smith MR, Fizazi K, Saad F, Mulders PF, Sternberg CN, et al. (2015). Abiraterone acetate plus prednisone versus placebo plus prednisone in chemotherapy-naïve men with metastatic castration-resistant prostate cancer (COU-AA-302): Final overall survival analysis of a randomised, double-blind, placebo-controlled phase 3 study. *Lancet Oncology*, 16, 152–160. [PubMed: 25601341]
- Ryan CJ, Smith MR, Fong L, Rosenberg JE, Kantoff P, Raynaud F, et al. (2010). Phase I clinical trial of the CYP17 inhibitor abiraterone acetate demonstrating clinical activity in patients with castration-resistant prostate cancer who received prior ketoconazole therapy. *Journal of Clinical Oncology*, 28, 1481–1488. [PubMed: 20159824]
- Saner KJ, Suzuki T, Sasano H, Pizzey J, Ho C, Strauss JF, 3rd., et al. (2005). Steroid sulfotransferase 2A1 gene transcription is regulated by steroidogenic factor 1 and GATA-6 in the human adrenal. *Molecular Endocrinology*, 19, 184–197. [PubMed: 15388788]
- SEER. (2017). Surveillance, epidemiology, and end results program. Retrieved from <http://seer.cancer.gov/statfacts/html/prost.html>.
- Selcer KW, Kabler H, Sarap J, Xiao Z, & Li P-K (2002). Inhibition of steryl sulfatase activity in LNCaP human prostate cancer cells. *Steroids*, 67, 821–826. [PubMed: 12231117]
- Shackleton CH, Kletke C, Wudy S, & Pratt JH (1990). Dehydroepiandrosterone sulfate quantification in serum using high-performance liquid chromatography/mass spectrometry and a deuterated internal standard: A technique suitable for routine use or as a reference method. *Steroids*, 55, 472–478. [PubMed: 2149221]
- Sharifi R, Bruskewitz RC, Gittleman MC, Graham SD, Jr., Hudson PB, & Stein B (1996). Leuprolide acetate 22.5 mg 12-week depot formulation in the treatment of patients with advanced prostate cancer. *Clinical Therapeutics*, 18, 647–657. [PubMed: 8879893]
- Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, et al. (2006). Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. *Cancer Research*, 66, 2815–2825. [PubMed: 16510604]
- Tamae D, Byrns M, Marck B, Mostaghel EA, Nelson PS, Lange P, et al. (2013). Development, validation and application of a stable isotope dilution liquid chromatography electrospray ionization/selected reaction monitoring/mass spectrometry (SIDLC/ESI/SRM/MS) method for

quantification of keto-androgens in human serum. *The Journal of Steroid Biochemistry and Molecular Biology*, 138C, 281–289.

- Tamae D, Mostaghel E, Montgomery B, Nelson PS, Balk SP, Kantoff PW, et al. (2015). The DHEA-sulfate depot following P450c17 inhibition supports the case for AKR1C3 inhibition in high risk localized and advanced castration resistant prostate cancer. *Chemico-Biological Interactions*, 234, 332–338. [PubMed: 25514466]
- Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, et al. (1995). Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. *The New England Journal of Medicine*, 332, 1393–1398. [PubMed: 7723794]
- Taplin ME, Montgomery B, Logothetis CJ, Bubley GJ, Richie JP, Dalkin BL, et al. (2014). 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. *Journal of Clinical Oncology*, 32, 3705–3715. [PubMed: 25311217]
- Tian Y, Zhao L, Zhang H, Liu X, Zhao L, Zhao X, et al. (2014). AKR1C3 over-expression may serve as a promising biomarker for prostate cancer progression. *Diagnostic Pathology*, 9, 42–48. [PubMed: 24571686]
- Trachtenberg J, Halpern N, & Pont A (1983). Ketoconazole: A novel and rapid treatment for advanced prostatic cancer. *The Journal of Urology*, 130, 152–153. [PubMed: 6306286]
- Tran C, Ouk S, Clegg NJ, Chen Y, Watson PA, Arora V, et al. (2009). Development of a second-generation antiandrogen for treatment of advanced prostate cancer. *Science*, 324, 787–790. [PubMed: 19359544]
- Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinänen R, Palmberg C, et al. (1995). In vivo amplification of the androgen receptor gene and progression of human prostate cancer. *Nature Genetics*, 9, 401–406. [PubMed: 7795646]
- Webster D, France JT, Shapiro LJ, & Weiss R (1978). X-linked ichthyosis due to steroid-sulphatase deficiency. *Lancet*, 1, 70–72. [PubMed: 74568]
- Woo LWL, Purohit A, Malini B, Reed MJ, & Potter BVL (2000). Potent active site-directed inhibition of steroid sulphatase by tricyclic coumarin-based sulphamates. *Chemistry & Biology*, 7, 773–791. [PubMed: 11033081]
- Wright JL, Kwon EM, Ostrander EA, Montgomery RB, Lin DW, Vessella R, et al. (2011). Expression of SLCO transport genes in castration-resistant prostate cancer and impact of genetic variation in SLCO1B3 and SLCO2B1 on prostate cancer outcomes. *Cancer Epidemiology, Biomarkers & Prevention*, 20, 619–627.
- Yamaoka M, Hara T, Hitaka T, Kaku T, Takeuchi T, Takahashi J, et al. (2012). Orteronel (TAK-700), a novel non-steroidal 17,20-lyase inhibitor: Effects on steroid synthesis in human and monkey adrenal cells and serum steroid levels in cynomolgus monkeys. *The Journal of Steroid Biochemistry and Molecular Biology*, 129, 115–128. [PubMed: 22249003]
- Yang M, Xie W, Mostaghel E, Nakabayashi M, Werner L, Sun T, et al. (2012). SLCO2B1 and SLCO1B3 may determine time to progression for patients receiving androgen deprivation therapy for prostate cancer. *Journal of Clinical Oncology*, 29, 2565–2573.
- Yen PH, Allen E, Marsh B, Mohandas T, Wang N, Taggart RT, et al. (1987). Cloning and expression of steroid sulfatase cDNA and the frequent occurrence of deletions in STS deficiency: Implications for X-Y interchange. *Cell*, 49, 443–454. [PubMed: 3032454]
- Yepuru M, Wu Z, Kyulkarni A, Yin F, Barrett CM, Kim J, et al. (2013). Steroidogenic enzyme AKR1C3 is a novel androgen receptor-selective coactivator that promotes prostate cancer growth. *Clinical Cancer Research*, 19, 5613–5625. [PubMed: 23995860]
- Yin YD, Fu M, Bokke DG, Heinrich DM, Denny WA, & Jamieson SMF (2014). The activity of SN33638, an inhibitor of AKR1C3, on testosterone and 17beta-estradiol production and function in castration resistant prostate cancer and ER-positive breast cancer. *Frontiers in Oncology*, 4, 159, 1–12. [PubMed: 24478982]
- Yu Z, Cai C, Gao S, Simon NI, Shen HC, & Balk SP (2014). Galeterone prevents androgen receptor binding to chromatin and enhances degradation of mutant androgen receptor. *Clinical Cancer Research*, 20, 4075–4085. [PubMed: 24874833]

- Zang T, Tamae D, Mesaros C, Wang Q, Huang M, Blair IA, et al. (2017). Simultaneous quantitation of nine hydroxy-androgens and their conjugates in human serum by stable isotope dilution liquid chromatography electrospray ionization tandem mass spectrometry. *The Journal of Steroid Biochemistry and Molecular Biology*, 165, 342–355. [PubMed: 27531846]
- Zang T, Taplin ME, Tamae D, Xie W, Mesaros C, Zhang Z, et al. (2017). Testicular vs adrenal sources of hydroxy-androgens in prostate cancer. *Endocrine-Related Cancer*, 24, 393–404. [PubMed: 28663228]
- Zhao XY, Malloy PJ, Krishnan AV, Swami S, Navone NM, Peehl DM, et al. (2000). Glucocorticoids can promote androgen-independent growth of prostate cancer cells through a mutated androgen receptor. *Nature Medicine*, 6, 703–706.

Fig. 1.

Adrenal and intraprostatic androgen biosynthesis. Conversion of cholesterol to DHEA is shown in the adrenal (*yellow box*). The conversion of DHEA to DHEA-SO₄ and its subsequent desulfonation by STS is not shown for simplicity. *4-AD*, 4-androstene-3,17-dione; *5-Adiol*, 5-androstene-3 β ,17 β -diol; *5-Adione*, 5 α -androstane-3,17-dione; *DHEA*, dehydroepiandrosterone; *HSD3B*, 3 β -hydroxysteroid dehydrogenase; *SRD5A*, steroid 5 α -reductase.

Fig. 2.
P450c17 inhibitors and their metabolites.

Fig. 3. Utilization of steroid sulfates in CRPC. *SLCO*, genes for organic anion transporter polypeptides; and *STS*, steroid sulfatase. Otherwise abbreviations are the same as in Fig. 1.

Mechanism 1

Mechanism 2

Fig. 4.

Irreversible inhibition of steroid sulfatase by sulfamate-based inhibitors. In mechanism 1 the sulfamate attacks the formylglycine residue at the active site. In mechanism 2, attack by an enzyme nucleophile gives rise to sulfamoylated enzyme.